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PARASITOLOGY

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VOLUME VII MAY, 1914 No. 1

SOME LARVAL TREMATODES FROM MILLPORT.

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(With Plate I.)

INTRODUCTION.

THE following work was done chiefly on material collected during a month's stay at Millport in August 1913 whilst working at the Marine Station. I have to thank Mr Elmhirst for much help in obtaining material and also for giving me the first specimens of the larval *Maritrema* encysted in *Ligia oceanica* which he discovered in the genital ducts of that Isopod. This is specially interesting as it is the first undoubted *Maritrema* larva known.

For the first specimens of the encysted Trematode from the Haddock's skin I am indebted to Dr Jas. Johnstone of Liverpool. From further material obtained at Millport this was found to be a larval *Opisthorchis*, again the first step towards the life history of any species of the genus as yet described[1].

In finding the young stages of *Parorchis acanthus* Nicoll, in the cloaca of the Herring Gull, together with the adults, it has been possible to identify the first larval stage as *Cercaria purpurae* sp. inq. (Lebour 1912) from *Purpura lapillus*, a specially interesting fact as it bears out the view that the genus *Parorchis* is closely allied to *Echinostomum*.

[1] Lühe, *Die Süsswasserfauna Deutschlands*, Heft 17, Trematodes, states that the young form of *O. felineus* (Riv.) encysts in the muscles of *Leuciscus idus* (L.) and *L. rutilis* (L.) undescribed at that time (1909).

The Life History of Parorchis acanthus *Nicoll.*

Parorchis acanthus (Nicoll 1907 *a*) is common in the cloaca of the Herring Gull at Millport and also at St Andrews where Nicoll got his type specimens. Among the adults in the Millport Gulls were several young forms which correspond so closely with the cercariae which I have named *Cercaria purpurae* from *Purpura lapillus* that there can be no doubt that it is the same species.

This cercaria is one of our commonest larval Trematodes and occurs in extraordinary abundance completely filling the spine of the mollusc when present and giving a curious swollen pale yellowish appearance to the digestive gland, so that it can at once be recognised. Some years ago Dr Nicoll told me he thought that this was the larva of *Parorchis acanthus* but it has not been possible to prove this until now. The intermediate host is still unknown in spite of constant searchings on my part in the animals living side by side with *Purpura*. I have found these cercariae in *Purpura* in the following localities: Fenham Flats, Budle Bay and Cullercoats in Northumberland (Fenham Flats being muddy ground with few rocks, Budle Bay rocky surrounded by sand, Cullercoats very rocky), Robin Hood's Bay in Yorkshire (rocky), the shores of Loch Ryan on the west coast of Scotland (muddy and sandy ground with stones) and on the coast round about Millport in Cumbrae (rocks surrounded by muddy sand). It occurs therefore in various habitats. The Herring Gull (*Larus argentatus*) is a constant feeder in all these places.

The animals almost always to be found with *Purpura* are Sea Anemones (*Actinia mesembryanthemum*), Green Crabs (*Carcinus moenas*), Rock Barnacles (*Balanus balanoides*), Limpets (*Patella vulgata*), Periwinkles (*Littorina littorea*, also frequently *L. rudis* and *L. obturata*) and very often Mussels (*Mytilus edulis* and *Modiola modiola*). All these I have examined for the encysted stages but with no success. I have also examined various worms from the mud or sand below the rocks and *Mya arenaria* from Millport and *Saxicava rugosa* from Robin Hood's Bay, all without success. The cercaria has a powerful tail which points to its leaving its first host and swimming to its second so that it seems unlikely that it ensysts in the *Purpura* or in an animal that feeds on *Purpura*. I should expect to find it in some animal living near and most probably in a mollusc as the cercaria closely resembles an *Echinostomum* cercaria, and those members of this genus whose complete life histories are known encyst in molluscs (*E. secundum* in *Mytilus edulis*,

E. leptosomum in *Scrobicularia tenuis*). The cercaria is developed in long rediae very like those of *Echinostomum* and its general form, suckers, alimentary canal, excretory system, tail and head spines (although the latter are somewhat different in form) remind us of this group. The excretory vesicle however does not extend into the tail, which is solid except for a sucker-like structure at the hinder end (Lebour 1912, pp. 446–7, Figs. 1–3, Plate XXIX). From these notes there seems to exist a close relation between *Parorchis* and the *Echinostomum* group, as is pointed out by Odhner who regards the genus as an aberrant form of an Echinostomid (Odhner 1913).

The length of a full-sized cercaria before it emerges from its first host is 0·64 mm. It has, when of this size, large spines on its body and spines round its head. It may be found, when full grown, either free in the digestive gland having come out of the redia, or in the mantle cavity near the gill, having migrated from the digestive gland on its way out of the body. This is as far as I can follow it until we find it again in the cloaca of the Herring Gull. The smallest worm from the cloaca measured 2·4 mm. (Plate I, fig. 1), so that a large increase in growth has taken place. The form of the body is identical, the body covered with thick spines dwindling towards the posterior end and a crown of spines forming a sort of collar interrupted ventrally by the oral sucker. The alimentary canal has the same form and likewise the excretory vesicle with its lateral branches, which however are much more conspicuous and complicated than in the tailed cercaria. The suckers have both increased in size especially the ventral sucker. In the smallest the oral sucker measured 0·16 mm. and the ventral 0·40 mm. (oral sucker and ventral sucker of tailed cercaria from *Purpura* 0·06 mm. and 0·09 mm. respectively). In larger forms the increase is greater. In specimens 2·8 mm. long the oral sucker was 0·32 mm. and ventral sucker 0·60 mm. across. Size in mature worm, oral sucker 0·50 mm., ventral sucker usually more than twice as great (Nicoll).

It is interesting to find this intermediate form as it indicates the first stage in the life history of *Parorchis acanthus* and also shows that at any rate in its early life it is exceedingly like *Echinostomum*. I have included it in this group when trying to classify the cercariae and these young stages help greatly in the elucidation of the relationships and proper systematic position of the adults.

A Larval Maritrema *encysted in* Ligia oceanica.

Specimens of *Ligia oceanica* were sent to me in October 1912 by Mr Elmhirst from Millport in which species he had found an encysted Trematode occurring abundantly in the genital ducts of both male and female. The *Ligiae* live just above high water mark in the tidal drift below the laboratory, generally under stones.

In both oviducts and vasa deferentia the cysts were scattered about, sometimes also occurring in the other organs, more particularly in the muscles. The cysts were either colourless or a bright yellowish brown, the latter probably being older, and inside each cyst was curled up a larval Trematode which undoubtedly is a species of the genus *Maritrema* Nicoll.

The cysts (Plate I, fig. 4) are oval and measure 0·36–0·45 mm. by 0·24–0·32 mm. and with a thick cell wall measuring 0·02 mm. in thickness. Each cyst has very much the appearance of a Trematode egg. I have called this worm *Cercaria ligiae* sp. inq.

Cercaria ligiae. Inside the cyst the curled-up larva is visible through the transparent wall. The oral and ventral suckers, alimentary canal and excretory vesicle are easily made out. The head and posterior end are folded in towards each other in the cyst. During the encysted stage the excretory organs of the posterior end have a peculiar structure. The vesicle is much dilated and full of conspicuously refractive granules. If by any chance the cyst is ruptured accidentally these granules always come out quickly from the excretory pore which is at the extreme posterior end. The granules are irregular in shape and perfectly clear.

The most successful way to get the worm out of the cyst is to leave it in normal salt solution over night. The larval worm will have emerged by the next morning and will be still living and can be examined alive. The refractive granules of the excretory system however will have all disappeared.

The whole body (Plate I, figs. 2 and 3), is quite colourless and transparent, very much flattened dorso-ventrally and with the sides having a tendency to fold over ventrally. The first two-thirds are covered with small spines and scattered about at much greater intervals are conspicuous unicellular glands. It is more or less tongue-shaped with the anterior end slightly more pointed than the posterior and usually narrow in the middle region. When contracted it is more oval. The length of the worm is 0·60 mm.–0·66 mm. and the breadth

0·28 mm.–0·32 mm., the greatest breadth usually being at the beginning of the posterior third of the body. The oral sucker is smaller than the ventral, measuring 0·04 mm.–0·06 mm. across, the ventral sucker measuring 0·06 mm.–0·08 mm. Both are circular in outline. The oral sucker is situated at the extreme anterior end of the body and leads by a circular aperture to the prepharynx which may be hardly recognisable when contracted but may measure as much as 0·04 mm. in length. The pharynx is large and muscular and measures 0·03 mm. in length and is rather longer than broad. The oesophagus is slightly longer than the pharynx and forks at a distance of 0·06 mm. in front of the ventral sucker. The intestinal caeca reach to the level of the posterior end of the ventral sucker. The ventral sucker is situated some way in front of the centre of the body and has a circular aperture.

The excretory system consists of a posterior vesicle which is Y shaped and gives off lateral ducts, the flame cells at the ends of the smaller branches being very conspicuous. The reproductive organs are far advanced in development. The testes lie just behind the ventral sucker and are large, lobed organs of a roundish form. Each measures about 0·13 mm. in diameter. Ducts arise from these but could not be followed; they spring from the anterior margins slightly to the inner side. The cirrus pouch lies in front of the ventral sucker and is well developed although the genital pore is not open and apparently the right end is not yet joined by the vasa deferentia. It is full of large nucleated cells and the vesicula seminalis is not formed. The ovary is conspicuous and lies on the right of the ventral sucker, none of it extending behind. It is roughly three-lobed and gives off a winding duct which loops behind the ventral sucker and then runs upwards, curving as it goes and enlarging to reach the left side of the cirrus pouch. The shell-gland complex could not be made out and probably is not completely developed. The vitellaria are of the typical *Maritrema* form and make a nearly complete wreath round the region behind the ventral sucker, running round the lateral margins and the posterior margin and then bending inwards in front of the testes and nearly joining, but leaving a small gap anteriorly. No vitellarian receptacle is as yet formed. The vitellaria consist of small rosettes of yolk material.

Hitherto no larval *Maritrema* has been known for certain, although *Cercaria oocysta* sp. inq. and *Cercaria pirum* sp. inq., both from *Paludestrina stagnalis*, seem to belong to this genus or to one closely allied (Lebour 1912 and Nicoll 1907 *b*). My suggestion that the habit of encysting in sporocysts (as these cercariae do) may distinguish this

genus from *Spelotrema*, has turned out to be erroneous as we see this present form encysting in a Crustacean.

So far the first larval stage of this species has not been found although much search has been made. The first host must almost certainly be a mollusc and the only mollusc living with *Ligia* below the laboratory at Millport is *Littorina rudis*. I have examined a great number of these but have not found a cercaria which certainly corresponds with this *Maritrema*. The *Littorinae* live under the same stones with *Ligia* as well as in small shallow pools and on the rocks close by, and also extend further down into the *Pelvetia* zone and even into the zone of *Fucus vesiculosus*. The *Littorina rudis* from all these parts contained several larval Trematodes the most frequent being the sporocysts and cercariae of what I believe to be *Spelotrema excellens* Nicoll, which are perhaps identical with the *Cercaria brevicauda* of Pelseneer from the same mollusc (Pelseneer 1906). This cercaria encysts in the Green Crab (*Carcinus moenas*), which does not live so high up as *Ligia* but does live with *Littorina rudis* lower down. I tried to find differences in these sporocysts and cercariae from the *Littorinae* living with *Ligia* but failed.

Three other species of cercariae were found in *Littorina rudis* living with *Ligia*:—*Cercaria linearis* Lespés. This occurred in one specimen together with the sporocysts of *Spelotrema excellens*. The cercariae are contained in sporocysts in the kidney whilst those of *S. excellens* are in the digestive gland. This is a new host for this species as hitherto it has only been known from the common Periwinkle *Littorina littorea* (Lespés 1857 and Lebour 1912).

Of the other two, one is a *Monostomum* cercaria in rediae, probably new, and the other is apparently identical with *Cercaria emasculans* Pelseneer (1906).

Cercaria emasculans Pelseneer. This cercaria occurred in sporocysts in the genital gland of *Littorina rudis* living with *Ligia* and also in that of *L. littorea* from Balloch Bay, Millport. It occurred more frequently in *L. rudis* which is the orginal host for it, *L. littorea* being new. The sporocysts are very like those of *Spelotrema excellens* and in many ways the cercaria resembles a *Spelotrema* cercaria. This made me think it might be the first stage of the *Maritrema*. The shape and size of the excretory vesicle however prevent my being in any way certain of this, although it is conceivable that the worm may grow very much larger without a proportionate growth in the excretory system. Some infection experiments were tried with this larval form and some *Ligiae* from

Plymouth which have not got this parasite, but these were unsuccessful.

The genital gland of *Littorina rudis*, when this cercaria is present, is white and very much swollen; in *L. littorea* it is orange-coloured although the colour only affects parts of the sporocyst and the tissues surrounding them, not the cercariae which are colourless.

The sporocysts (fig. 6) are long and thin-walled, 0·40 mm.–0·80 mm. long and 0·20 mm.–0·35 mm. broad. All were full of cercariae in various stages of development.

The cercaria (figs. 5, 6) is extremely active with a long tail which is continually in motion and the whole worm is so constantly changing its shape that its structure is very difficult to see when alive. The body measures 0·38 mm. in length without the tail, but can be much shorter or longer when contracted or expanded. The tail is nearly the length of the body. The breadth of the body is about a third of the length. The whole surface is covered with minute spines dwindling posteriorly. The oral sucker measures 0·03 mm. across and is circular with a circular aperture. On its dorsal surface is a single straight stylet measuring in length not quite half the width of the sucker. The ventral sucker is peculiarly prominent protruding as a great bulb from the ventral surface. It is situated almost exactly in the centre of the body and is the same size as the oral sucker or slightly larger. The aperture which is round is set at an oblique angle to the body.

The oral sucker leads to a somewhat obscure alimentary canal with no apparent pharynx (Pelseneer however shows a pharynx in his figure so my specimens may be younger than his). In some the intestinal caeca could be made out, with the fork well in front of the ventral sucker and the caeca reaching to just behind the level of the ventral sucker and slightly swollen at the ends, reminding one very much of the intestine of the encysted *Maritrema*.

The excretory vesicle is Y shaped, the forks reaching to the level of the anterior margin of the ventral sucker. The tail is very transparent and extremely contractile with striations both transverse and longitudinal, the transverse being the more conspicuous.

I think it is possible that this cercaria may prove to be the first stage of the *Maritrema* from *Ligia* but this is as yet not at all certain.

With regard to the adult stage of this *Maritrema* it does not agree exactly with any known species and is probably a new one.

The species of *Maritrema* all inhabit birds and Nicoll (1907) describes three British species occurring in the Dunlin (*Pelidna alpina*),

Ringed Sand Plover (*Aegialites hiaticula*), Black-Headed Gull (*Larus ridibundus*), Herring Gull (*Larus argentatus*), Oyster Catcher (*Haematopus ostralegus*), and Redshank (*Totanus calidris*). Of these the only one which has the ventral sucker greater than the oral is *Maritrema humile* from the Redshank but in other ways this does not agree with our species.

Jägerskiöld (1908) gives a summary of the characters of *Maritrema* and divides up the species of the genus into groups, also describing some new species. Of these his *Maritrema subdolum* from the Common Sandpiper (*Actitis hypoleucos*) comes very near the present species, although I do not think they are identical.

At Millport I have examined many birds for the adult worms but as yet without success.

A larval Opisthorchis *encysted in the skin of Gadoids.*

In September 1912 Dr Jas. Johnstone kindly sent me pieces of a Haddock (*Gadus aeglefinus*) the skin of which was spotted at frequent intervals with black, each spot marking the presence of an encysted larval Trematode. Owing perhaps to the preservative it was difficult to make out the structure of these worms, the cyst being extremely tough and difficult to break through. Enough was seen however to show that it was a Distomid. Gamble and Drew (1912) noticed the same appearance in a Whiting (*Gadus merlangus*) but in this the parasite was identified by them as probably a species of *Holostomum*. It is evident that many Trematodes cause the segregation of pigment granules in the neighbourhood of the cysts. Nicoll (1907 *c*) found an encysted Trematode in a *Cottus bubalis* also with black pigment. Williamson (1911 *a* and *b*) notices the same thing in the "Spotted Haddock" but believes his specimens to be *Gasterostomum gracilescens*, *i.e.* the same species that inhabits the nerves of that fish. There are also several examples from American fish, notably the Trout (Linton 1911, p. 252).

It was not until this summer (1913) that I managed to study a Trematode in the skin of a Lythe from Millport which evidently belongs to the same species as those in the Haddock sent to me by Dr Johnstone. Lythe (*Gadus pollachnis*) and Saithe (*Gadus virens*) are frequently spotted in this way at Millport and they come quite close to the shore so long as the water is fairly deep. In Jan. 1914, Mr Elmhirst sent me portions of the skin of a Cod with similar spots which contain the same worm.

The fish in question was spotted at infrequent intervals dorsally, some spots being below the lateral line and more above it, many occurring near and on the fins. A fair number of cysts were found in the muscles below the skin and even here there was a small amount of pigment present. The fish was quite healthy and seemed not at all inconvenienced by the cysts. The skin was quite clean and to a casual observer the spots might have seemed natural to the fish.

From its habit of attracting pigment I propose for this worm the name *Cercaria chromatophila*.

Cercaria chromatophila sp. inq. The cyst is oval and measures 0·36 mm. × 0·26 mm. which is very much the same size as Williamson's cysts (1911). Round it is deposited the blackish-brown pigment either completely covering it or leaving a small area on the outside uncovered. The pigment is arranged in closely aggregated star-like masses close together near the cyst and gradually dwindling as they get farther away from it (Plate I, fig. 9). A fibrous covering containing the pigment can be removed from the cyst by careful manipulation with needles and the worm is then seen coiled up inside. The cyst is formed of two coats both colourless and transparent, the first thick and extremely tough and elastic, the second, immediately internal to it and closely pressed to its walls, is very thin and easily ruptured. Great difficulty was experienced in opening the cyst without damaging the worm; the most successful method was found to be to soak the cyst in normal salt solution for a day and then under a dissecting microscope prick it with a fine needle. By this means it was possible to get the worm to emerge still alive (figs. 7–8).

Fully extended it measures 0·70 mm. in length, the greatest breadth being 0·24 mm. at the anterior third of the body which is pointed at each end but more blunt anteriorly, colourless and transparent.

It is covered completely with small spines which dwindle posteriorly although they do not entirely disappear.

The oral sucker is circular with a circular aperture at the extreme anterior end. It measures 0·06 mm. across, the aperture being 0·03 mm. across. A very small prepharynx 0·01 mm. in length leads to a broad and muscular pharynx 0·03 mm. long and this leads to the oesophagus 0·03 mm. long which branches into two long caeca which are narrow and bend inwards in front of the ventral sucker, curve outwards again and inwards with the tapering of the body and end at the extreme posterior end. Inside the intestine are numerous colourless refractive granules. The ventral sucker is situated very far back at the junction between

the middle third and posterior third of the body, and 0·23 mm. from the intestinal fork. It measures 0·04 mm. across. Just in front of it the oviduct runs up for about 0·02 mm. beyond it.

The excretory vesicle is **Y** shaped, opening at the posterior end of the body. The stalk measures 0·11 mm. in length, each fork measuring 0·05 mm. The vesicle also contains a refractive substance which is very conspicuous in the encysted worm.

The testes are oval and occur obliquely one on each side of the excretory vesicle, the left in front of the right. Each measures 0·04 mm. × 0·02 mm.

It is evident from the arrangement of the organs, especially the excretory vesicle and the testes, that this is a species of *Opisthorchis* as, so far as the anatomy can be made out, it agrees very well with the diagnosis of that genus (Looss 1899, p. 564), although it is described as without spines except in one species. It is interesting to note that the encysted worm found by Nicoll in the eyes etc. of *Cottus*, although not fully described, agrees in all the points he mentions with this worm.

The final host will in all probability be found to be a fish-eating bird. The group to which *Opisthorchis* belongs includes forms from mammals, birds and fishes, chiefly birds. Odhner (1912) describes an *Opisthorchis* from the gall bladder of the Electric Eel (*Gymnarchus niloticus*). Braun (1902) describes one from a Heron (*Ardea stellaris*) from Brazil which is much like the present worm. So far as I am aware no adult *Opisthorchis* has as yet been recorded from Britain and this is probably a new species. It seems to be the first record of a larval form of this genus and is interesting on that account.

DESCRIPTION OF PLATE I.

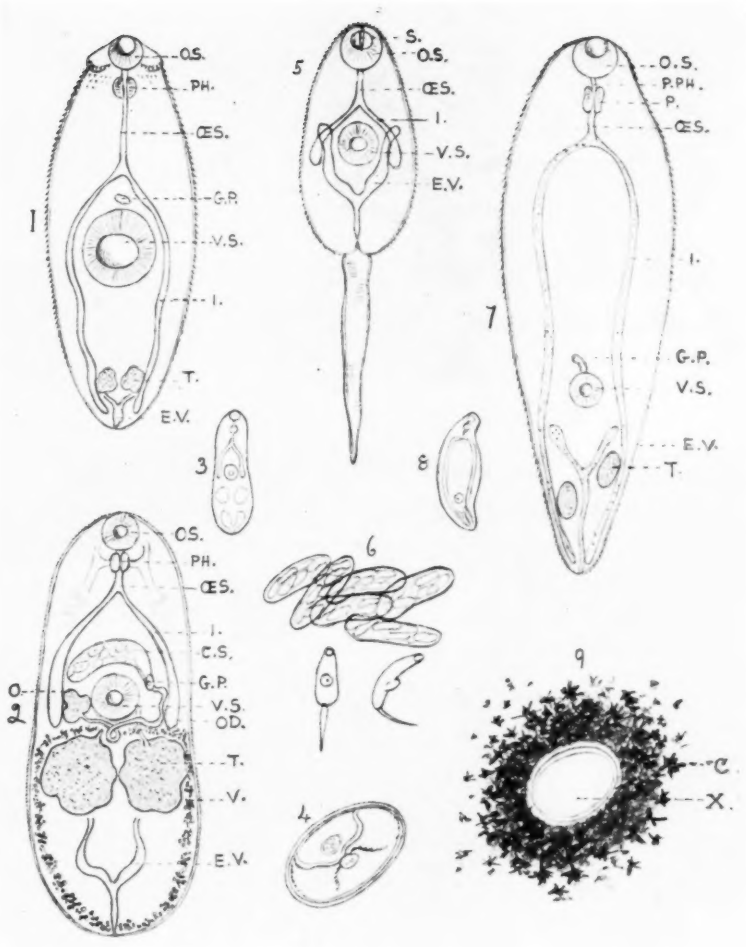
The following letters apply to all the figures:

C. pigment
C.S. cirrus sac
E.V. excretory vesicle
G.P. genital pore
I. intestine
O.S. oral sucker
ŒS. oesophagus
OD. oviduct
O. ovary
P. pharynx
PPH. prepharynx
T. testis
V. vitellaria
V.S. ventral sucker
X. worm in cyst

Figs. 1, 3, 6 and 8 are on the same scale.

Fig. 1. Young stage of *Parorchis acanthus* Nicoll, from cloaca of Herring Gull. Size 2·4 mm.

Fig. 2. *Cercaria ligiae* sp. inq., larva pressed out of cyst. Size 0·65 mm.



M. V. Lebour, del.

Fig. 3. The same under low power.
Fig. 4. The same encysted. Size 0·36 mm. × 0·24 mm.
Fig. 5. *Cercaria emasculans* Pelseneer. Size 0·38 mm. without tail.
Fig. 6. The same showing sporocysts.
Fig. 7. *Cercaria chromatophila* sp. inq. Size 0·70 mm.
Fig. 8. The same under low pressure.
Fig. 9. The same in cyst with surrounding pigment. Size of cyst 0·36 mm. × 0·26 mm.

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SHORT DESCRIPTION OF THE LARVA OF *LOPHOSCELOMYIA ASIATICA*, LEICESTER 1905, AND NOTES ON THE SPECIES.

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(With 3 Text-figures.)

THE larva of Leicester's bamboo-breeding anopheline "*asiatica*" as Stanton says (1912) has not been previously described, although Leicester himself mentioned in his monograph that it was characterised by the possession of a very conspicuous black marking on the thorax shaped like a Maltese Cross; so having recently obtained a batch of seven of these creatures from a cut-bamboo at Ginting Simpah, the pass between the Native Malay States of Selangor and Pahang which is at an altitude of 1500 feet, I will attempt to fill the deficiency in our knowledge, adding as well a few notes on the biological side of the subject.

Description of Larva.

As Leicester says, the *naked eye appearance* of the larva is very striking, for on an almost uniformly old-gold colouration is this conspicuous Maltese Cross marking on the thorax, and also another very distinct black marking about half-way down the abdomen. The head is tinged with a deeper colour than elsewhere. No other Malayan anopheline larva as yet described could be possibly mistaken for it, although Colonel Alcock's new species *wellingtoniana*, in view of the striking similarity in the adult stage between it and *asiatica*, might ultimately be found to present the same appearance. The larva of *asiatica* is large, and resembles in shape the larvae of the *Myzorhynchus* group of species.

The *microscopic appearances* (see Fig. 1) which I here give were observed on the living larva, and also on some which had been preserved in a mixture of rectified spirit 40 % and formalin 10 %. The colouration of the larva is well preserved in the latter fluid.

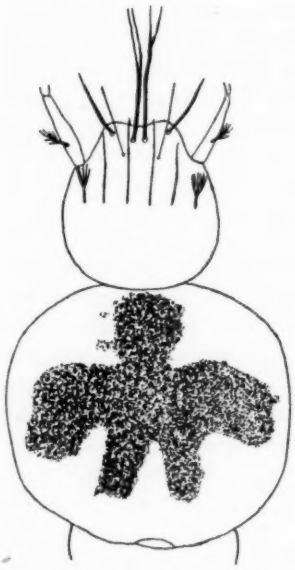

Fig. 1.

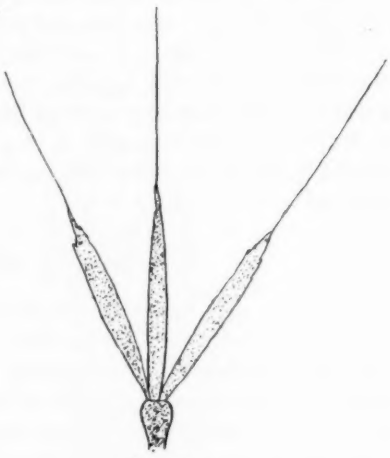

Fig. 2.

Fig. 3.

The larva of *Lophoscelomyia asiatica*.

Fig. 1. The head and thorax. Fig. 2. A palmate hair.
Fig. 3. The 4th abdominal segment.

Structure.

Head. Clypeal hairs. (i) *Anterior.* The internal hairs are long, stout, and close together, as in members of the *Myzorhynchus* group, and are bifurcate about their middle. (ii) The *external* hairs are about half the length of the internal, stout, projecting forwards and outwards, and simple. (iii) *Posterior.* These hairs are a little longer than the external ones and are simple and delicate.

The *frontal hairs* are six in number as usual, but only the two external ones are branched. The four internal hairs are very delicate.

The *antennae* each possess a branched hair, as in *barbirostris* and *sinensis*, and the Indian species *lindesayi*, but it is situated on the *external* side of the antenna.

Thorax. There are no palmate hairs on the thorax. Under the microscope the black Maltese Cross has the appearance represented in the figure (Fig. 2). The posterior limb of the Cross is bilobed.

Abdomen. In this region of the body the palmate hairs are slightly developed on the 2nd segment, well developed from the 3rd to 7th segment. They are large and pigmented. Each leaflet is long and rather slender, terminating in a long filament without any very jagged shoulder. There is a dense black spot on the dorsum of the 4th segment (Fig. 3).

Note on the imago.

Leicester does not lay much stress on the striking appearance presented by the cream-coloured ventral parts of the thorax, particularly the coxae, trochanters, and proximal ends of the femora. The silver spot at the distal end of the mid-femur is very conspicuous. I have not been able to see any colour-spot on the wing fringe at the apex, as reported by Leicester, and in this connection I may also mention that I have seen *albotaeniatus* with an apical fringe spot, although Leicester says that it has not got one. It seems, therefore, that this character is somewhat variable.

Biological note.

One larva was observed to be in apparently a full-grown state for about four weeks without pupating. I therefore obtained a cut-bamboo and put the larva in it from the bottle in which it had been kept, but after another week it being still in the same state, I placed it in a cool dark corner of the house instead of on the bright verandah and the next morning it had pupated, and two mornings afterwards the imago had emerged. It seems, therefore, that bright light exercises a deterrent influence on the late development of this species. Indeed the Malayan jungle, which is its natural habitat, is always densely shady. I noticed that the larvae placed in the cut-bamboo always took refuge at the dark end. Light however *seems* to exercise no inhibitory influence on the early growth of the larva, for three very minute ones grew very well on the bright verandah until they were quite large. These three pupated after 35, 41 and 43 days, respectively.

Note on the species.

There can be no doubt that this species, together with the members of James and Liston's genus *Patagiamyia* and of Blanchard's genus *Myzorhynchus*, forms one very distinct group in the Old World. In fact the group is so distinct that it is difficult to say to what other species of anopheline it has any affinity. It is characterised, firstly in the larva, by the presence of an antennal hair in all known species, and the internal clypeal hairs are close together; secondly, in the adult, by the possession of a tuft of scales on the patagia, and a very uniform wing-pattern, namely, a golden spot on the costa at the junction of the subcostal vein and another at the junction of the first longitudinal with the costa, this involving the upper branch of the second longitudinal. Another uniform character is the obovate shape of the wing-scales. Moreover the general facies, although incapable of exact definition, is very characteristic. All the members are jungle breeders.

On the other hand James and Liston appear to see affinities between their *Patagiamyia* group of these species and the *Anopheles* group (*sensu* Meigen), and also between their *Myzorhynchus* group (*sensu restricto* James and Liston) and the *Christophersia* group. There may be this affinity although it is difficult to see it, but in any case there seems to be very much more affinity between those authors' *Patagiamyia* and *Myzorhynchus* species than between *Anopheles* on the one hand and *Christophersia* on the other, and this similarity should be reflected in a grouping under one name.

It is another question whether it is advisable to form a sub-group for any of these myzorhynchoid species or to take them all as belonging to the genus *Myzorhynchus* Blanchard. But in the latter case the original definition of the genus should be emended, for at present it could not apply to *asiatica* or *umbrosus*, for example. Certainly *asiatica* and *wellingtoniana* could be separated from the others in one group by reason of the well-marked tufts of scales on the femora, but this might be a conventional grouping, for I have seen a *barbirostris* with quite a well-marked clump of scales at the distal end of the femora; the scaling of the last abdominal segment of *asiatica* is not a distinctive character, as *umbrosus* sometimes is so scaled. If the sub-grouping however is kept, the definition of it will have to depend on the femoral scale-tufts.

With regard to the other species of the group it seems difficult to know where to draw the line: James and Liston's division into *Patagiamyia* and *Myzorhynchus* cannot stand, as we have in Malaya a species

which presents at different times the distinguishing (*sic*) characters of both genera. This species is *sinensis* as I have shown elsewhere (1913).

My conclusion therefore is that it would be difficult to divide up these species into sub-groups at all, and that they should be referred to the genus *Myzorhynchus* Blanchard, the definition of which however should be emended.

The affinities of these Malayan myzorhynchoid species are possibly reflected by the following order.

barbirostris	ventral scale tuft on 8th abdominal segment,
sinensis	„ „ „ sometimes present, or segment glabrous,
albotaeniatus	last abdominal segment glabrous,
umbrosus	„ „ „ sometimes with flat scales,
wellingtoniana	„ „ „ with flat scales,
asiatica	„ „ „ with flat scales.

It remains now to discover and describe the larvae of *punctulata* and *wellingtoniana* which are the only Malayan anopheline larvae not known.

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BLOOD PARASITES FOUND IN MAMMALS, BIRDS AND FISHES IN ENGLAND.

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(With Plates II–V.)

During an investigation, extending over several years, of the blood of numerous animals found locally, I have met with numerous parasites most of which are well known, but some, at least to me, are new.

Among the latter were especially the following:

1. Immature forms of *Trypanosoma vespertilionis* in *Vesperugo pipistrellus.*
2. Possible schizogonic forms of the *Leucocytozoon* of the thrush, found in the peripheral and heart blood and in that from the lung.
3. *Haemogregarina* in the blood of the field mouse and field vole.
4. Intracorpuscular bodies of the nature of *Piroplasma* in the blood of the field mouse and water vole.
5. *Spirochaeta* in the blood of two species of bats, the Pipistrelle and the Noctule.
6. *Toxoplasma* and *Haemogregarina* in blood and organs of the squirrel.

It is quite impossible for me with such limited literature at my disposal to determine whether any of these represent new species, but I have given a brief description and have added photomicrographs of each so that they may be identified.

It is customary to decry the mere hunt for, and the adding of names to, new parasites, and although I would to some extent plead guilty to this indictment, my investigations were primarily undertaken to try and

determine whether trypanosomes, for example, passed any of their life history as intracorpuscular bodies.

At least one thing was acquired by these examinations and that is some degree of efficiency in detecting new bodies foreign to normal blood.

With regard to the methods used there is little to say. Films of blood obtained from the peripheral vessels, the heart and in some cases from the internal organs were in all cases immediately fixed with the vapour of 1% osmic acid, and then allowed to dry. Drying was expedited by using slightly warm slides or by waving the films in the air.

These were stained either by Leishman's or, after fixation with absolute alcohol, with Giemsa's stain. A few were first covered with Leishman's solution for about a minute and then stained in the ordinary way with Giemsa.

Latterly I found that Giemsa's new rapid method gave by far the best results. The stock solution of Giemsa's stain is diluted with equal volumes of Merck's pure methylic alcohol and this is used very much like Leishman's method, only very much more neutral distilled water was added. Parasites which stain indifferently with Giemsa's stain used in the ordinary way after fixation with absolute alcohol, are extremely well stained by Giemsa's new method.

Preparations were invariably mounted in Parolein, as I (1911) have described elsewhere. In this medium the parasites are not liable to fade, and on account of the low refractive index and total absence of colour of Parolein, or liquid Paraffin, their minute structure is rendered more visible.

TRYPANOSOMES.

Trypanosomes, mature and immature of the Pipistrelle.

I have examined the blood of 25 bats belonging to three species and have found the following parasites.

Trypanosomes were present in three out of 20 common small bats or Pipistrelle (*Vesperugo pipistrellus*), 13 of which came from Reading. The three infected Pipistrelles were amongst those kindly sent me by Mr Ed. and Miss Blatch of Reading, and I take this opportunity of expressing my thanks to them for their kindness in procuring those and other specimens.

In one of these Pipistrelles the infection was severe and stages in development were seen (May 1906), in another (1913) the trypanosomes

were fairly numerous and were associated with the presence of very numerous intracorpuscular parasites, *Achromaticus vesperuginis*, and in the third (1913) the trypanosomes were exceedingly rare, only five being found in 20 films which, however, contained a heavy infection of *Achromaticus*.

Achromaticus vesperuginis was found in seven Pipistrelles, two of which showed a heavy infection, two were moderate and in three the parasites were very scanty.

Haemamoeba, pigmented intracorpuscular parasites, were found in one Pipistrelle caught locally.

Spirochaeta I found in very small numbers in one Pipistrelle together with a large number of *Achromaticus*, and in one of the large short-eared bats, the Noctule (*Vesperugo noctula*), in which it was associated with a *Filaria*.

Two long-eared bats (*Plecotus auritus*) caught locally were examined but no parasites were discovered.

Thus in the blood of 9 out of 25 bats parasites were found, and with one exception the infected bats came from Reading. This does not necessarily mean that the bats caught locally are less infected, but that most of the specimens sent from Reading, 13 in number, were examined when I had become more skilled in detecting some of these not easily recognised parasites.

Trypanosoma vespertilionis (Battaglia).

In one of the Pipistrelles sent from Reading (May 1906) I found a very large number of trypanosomes both in the immature and adult stages.

The proportion of the different forms varied remarkably in five films made, as far as I can remember, from the blood taken from the heart, but I counted 372 immature to 428 fully grown parasites, i.e. 46·5 % were immature developmental trypanosomes and 53·5 % adult types.

Immature Trypanosomes. These are for the most part somewhat pear-shaped, some oval and a few almost round. (Plate II, figs. 9, 10, 11.)

Each possesses a nucleus, centrosome and one or more flagella. The protoplasm is slightly granular and stains blue. The nucleus, which is about 2 μ in diameter, is situated either in the centre or more generally somewhat towards one end of the organism. It is usually round, and shows little structure, except that it appears to consist of a number of

granules arranged in a radial manner. The centrosome is usually round, sometimes oval or rod-shaped, and stains very deeply with Giemsa's stain. Its position in the body varies according to the size, and therefore probably the age, of the parasite. In the smallest forms it lies near or actually in contact with the nucleus, whilst in the larger forms it is placed somewhat near the rounded end of the pear-shaped structure. It usually measures about 1 μ in diameter.

A flagellum starts from, or more generally near, the centrosome and is in some cases thickened or V-shaped at first. It is usually about 4 to 5 μ in length, of which 3·5 μ is free. In a few there are two flagella, originating from or near a single centrosome, and these are distinctly separate in their course inside and outside the body.

The average size of the immature trypanosome was 6·3 μ in length and 2·7 μ in breadth, but larger forms measuring 7·5 μ by 4·5 were seen, containing two nuclei and two centrosomes placed side by side with two flagella to each centrosome. (Plate II, fig. 9.)

One circular body measuring about 5 μ in diameter was found in which there were eight nuclei arranged in a ring lying towards the margin of the cell. This showed no evidence of flagella, and as unfortunately all these preparations were mounted in so-called neutral canada balsam they have faded too much to say whether there was any indication of a centrosome, but camera lucida drawings made at that date show no such signs.

Adult Trypanosomes. These somewhat resembled *T. lewisi* but were very much smaller, possessed a relatively long flagellum, and were generally very much curled up, so that the posterior end came in contact with the anterior end, and they resemble to some extent the letter Q. (Plate II, fig. 8.)

During life they are very active, coiling and uncoiling, but do not make much progress. The nucleus is situated somewhat nearer the anterior than the posterior or aflagellar end; it shows a radiate structure. The centrosome is generally round and is placed very near the posterior extremity, but it is not actually at the tip, as the extreme posterior end terminates in a very fine sharp point which is 1 to 1·5 μ from the middle of the centrosome. The latter is relatively large, and in some cases seems to project beyond the margin of the body proper.

From or near the centrosome the border of the undulating membrane starts, and runs with few turns to the anterior extremity, becoming free as a somewhat long flagellum which is about two-thirds the length of the body.

The body of the trypanosome stains deeply blue with Giemsa and in some preparations contains vacuoles. It terminates abruptly behind, ending in a very fine point, whilst anteriorly it gradually becomes thinner.

The following were the measurements of twenty adult *T. vespertilionis*:

Total length	19·3 μ	Posterior end to centre of nucleus	7·0 μ
Length of body	11·7	Width of body	2·0
Length of flagellum	7·6	Nucleus width	1·0
Posterior end to middle of centrosome	0·75 to 1·5	Nucleus length	1·7

Degenerate Trypanosomes. In the Pipistrelle, examined more recently (1913), among the normal trypanosomes were an exceedingly large number of degenerate parasites. These appeared either as swollen transparent trypanosomes with large faintly stained nuclei and centrosomes, or what was more frequent, as merely the remains of a flagellum with centrosome and some débris of the nucleus and body.

Another point of interest was that in numerous films made from this Pipistrelle the parasites were invariably much more numerous in the first quarter of an inch of the film. Here they could readily be found, whilst in the remaining part of the film, which is usually much clearer and more uniformly spread, it required a very prolonged examination to detect a single trypanosome. This peculiarity is also more noticeable in the case of *Spirochaeta vesperuginis* which is subsequently described.

Trypanosoma talpae.

I have met with trypanosomes in the blood of five out of eighteen moles (*Talpa europaea*). They were always present in small numbers, and although fairly large in size they are not easily detected in stained films. The reason of this I think is that however well the preparation may be stained with Giemsa's solution, the parasites are seldom coloured blue; for the most part the whole of the trypanosome assumes a reddish transparent tint.

When found in fresh preparations they were seen to move rapidly with the flagellum in front.

I met with one which seemed to be very much larger than the ordinary form, but on careful examination this proved to be two individuals attached by their posterior extremity, and might have been a dividing form, although in stained films I have met with no signs of division.

In two out of the five moles, *T. talpae* was associated with the intracorpuscular bodies *Elleipsisoma thomsoni*, whilst Graham-Smith's bodies were commonly present.

The general shape of the body of the trypanosome is that of an elongated spindle, both the anterior and posterior extremities being drawn out to a sharp point, the aflagellar end being particularly pointed.

The protoplasm, as just mentioned, stains very faintly with the basic colour, and often contains chromatoid granules especially in the posterior half. (Plate II, fig. 6.)

In some trypanosomes structures were seen resembling a miniature trypanosome and at first I was inclined to regard them as such, but I sent a specimen to my friend Mr E. M. Nelson for examination and he reported that they might possibly be young trypanosomes, but in all probability the *T. talpae* were not viviparous and the structures might represent folds in the body.

The nucleus is small and is situated about the middle of the body as regards its long axis, but nearly always to one side, whilst the centrosome is placed on the other side of the parasite. The distance of the centrosome from the posterior extremity was considerable, measuring on an average 8·7 μ, but this might reach 10 μ.

The average measurements of five *T. talpae* were as follows:

Total length	28·3 μ	Posterior end to middle of nucleus	13·4 μ
Length of body	22·9	Width of body	4·9
Length of flagellum	5·4	Nucleus length	2·1
Posterior end to middle of centrosome	8·7	Nucleus breadth	1·3

Centrosome diameter 0·5 μ.

Quite recently I have found in the blood of a mole some extremely long ribbon-like forms of trypanosomes associated with the ordinary type of *T. talpae*. These were about 38 to 40 μ in length, of which the body measured 32 to 33 μ and the free flagellum about 6 to 7 μ. They were especially remarkable for the extreme length of the posterior extremity which has about the same width as that of the body, 2 μ, but gradually tapers off, and measures 17 to 18 μ from the centrosome to the posterior extremity. The centrosome actually lies in the centre, or even nearer the anterior than the posterior end of the body, and the nucleus is usually situated very near the anterior extremity.

These forms probably bear the same relation to *T. talpae* as *T. longocaudense* does to *T. lewisi*.

Degenerate Trypanosomes. In the blood of moles that had been dead some hours I not infrequently met with structures which were obviously remains of trypanosomes. These bodies were round, transparent structures staining very faintly with Giemsa, and containing a few chromatoid granules. Towards the margin one could make out a part faintly coloured red, and at times a spot representing a centrosome, whilst in a few distinct signs of a flagellum were present. It seemed as if the trypanosome had rolled itself up into a ball or had become swollen into a cyst-like body.

Trypanosoma lewisi.

The number of rats (*Mus decumanus*) found infected varies considerably in this locality, but I should expect to find trypanosomes in the blood of one or two out of five fully grown rats. I have thought that the parasites were not so frequently present in young rats. (Plate II, fig. 7.)

The form of *T. lewisi* found is very constant, although I have noticed that in one case, a rat which was also infected with haemogregarines, all the trypanosomes were larger than usual.

I have examined the blood of a very large number in an endeavour to find developmental forms in the naturally infected common rat, but have entirely failed.

I have nothing to add to the well known description of this trypanosome, except that in well but perhaps lightly stained preparations the centrosome almost invariably shows a line through the centre, thus ◖◗. My friend Mr E. M. Nelson first pointed this out to me. It is by no means peculiar to *T. lewisi* but is seen in many species. It requires a good lens, critical illumination and above all a trained eye to detect it.

Pulmonary Cysts in the Rat.

In smears of the lung taken from a very large rat which had been much mauled by a dog, I found small bodies about the size of a red corpuscle, containing eight blue stained small merozoites or vermicules. They were very infrequent, not more than 20 being found on a $\frac{3}{8}$ inch coverglass. (Plate II, figs. 12 and 13.)

They are generally round, sometimes oval, rarely pear-shaped, and stain a pale blue colour with Giemsa. They varied in size but on an average measured 5 to 6 μ in diameter. Each cyst contained eight small bodies which stained a deep blue colour with Giemsa, and had

a round or oval nucleus. Sometimes a structure resembling a centrosome was also seen.

The vermicules or merozoites measured about 0·75 μ by 1·25 μ.

In a few specimens a fine line could be detected reaching from the nucleus to the centrosome.

In addition to the enclosed vermicules a number of similar bodies were seen free, but besides these two forms there were bodies irregularly placed in a bluish matrix, having many of the characteristics of *Leishmania*. Smears made from the liver, spleen, and bone marrow were negative, and no trypanosomes could be found in the peripheral or heart blood.

Carini regarded them as forms of schizogony of *T. lewisi* and stated that they were derived from trypanosomes which had become encysted.

Delanoe (1912) found these cysts, which average 5 μ in diameter, containing eight vermicules, more frequently in old than in young rats. He says that of 22 adult rats, cysts of Carini were found in all, but none were infected with trypanosomes.

He concludes that they represent a new parasite of rats, which has nothing to do with *T. lewisi* and terms them Pneumocysts of Carini, and regards them as belonging to the Coccidia.

Trypanosoma cuniculi.

I have not had many opportunities of examining the blood of rabbits except those which have been dead some time, but occasionally I have met with *T. cuniculi*, always in small numbers, sometimes associated with Coccidia. The trypanosome closely resembles *T. lewisi* but differs from the latter in that it is generally smaller, the posterior extremity is somewhat shorter and thinner, the body shorter, whilst the flagellum is longer.

The following figures give the average measurements of *T. cuniculi*:

Total length	27 μ	Posterior end to middle of nucleus	11 μ
Length of body	17	Width of body	1 to 1·5
Length of flagellum	10	Length of nucleus	2
Posterior end to middle of centrosome	2·5 to 3	Width of nucleus	1

Trypanosome of Cow.

In the blood of a cow suffering from Redwater Fever I found a huge trypanosome, and in the same film another degenerated or distorted example. The parasite was in the form of an almost perfect **S**, being

thicker in the middle and tapering towards both extremities. As a full description of this trypanosome has been given previously (1913) I will merely add the measurements which were as follows.

Total length, 98 μ; length of body, 88 μ; free flagellum, 10 μ; distance from the posterior extremity to kinetonucleus, 37 μ, and to the centre of the nucleus, 44 μ; distance between trophonucleus and kinetonucleus, 5·5 μ; diameter of body at trophonucleus, 6 μ, together with the undulating membrane, 9·5 μ.

TRYPANOSOMES OF BIRDS.

During the course of a somewhat extensive examination of the blood of birds I have met with trypanosomes in the blackbird (*Merula vulgaris*), the thrush (*Turdus musicus*) and the jay (*Garrulus glandarius*). In 1904 I met with one trypanosome in a blackbird which I have described elsewhere (1906). In the thrush both large and small trypanosomes were found, in the blackbird and jay only the large variety has so far been seen. I have met with about 150 trypanosomes in blackbirds, about 100 in thrushes and about 40 in the jay.

Trypanosomes of the Blackbird.

These vary very much in numbers. In a cock bird killed July 10, 1911 they were the most numerous; 44 well spread *blood* films were examined over an area of a $\frac{3}{4}$ inch coverglass and 40 of these were found to contain 124 parasites, four films proved negative, the average number of trypanosomes found on each film was about three and the largest number seen on a single film was 13. Several films of bone marrow were examined but with negative results, whilst a single parasite was found in a lung and liver smear. The trypanosomes in this bird were associated with the round form of *Leucocytozoon*.

The trypanosomes may for convenience of description be divided into two classes which I have termed the Fusiform and the Piriform in outline. Although these may merely represent different aspects of the same organism, they certainly have some distinctive features. The fusiform or spindle-shaped parasites stain a much deeper blue colour, they almost invariably show myonemes or contractile fibrils, they are longer, narrower, and possess a longer flagellum. The piriform are roughly pear-shaped, they stain a much lighter colour, they are shorter and broader, never show myonemes, but the protoplasm is generally somewhat granular.

The Fusiform Trypanosomes. The body is long and is generally about five times as long as it is broad. The greatest width is about the middle third, and the body usually tapers to a fine point at each end. (Plate II, fig. 1.)

The protoplasm stains a deep blue colour and in or under the periplast very distinct myonemes are seen. These run as longitudinal striations and are stained bluish or purplish alternating with lighter streaks, and with critical illumination they can be resolved into a dotted appearance. They continue over the nucleus and for the most part run parallel on the two surfaces of the body, at other times they cross giving the appearance of lattice work. Chromatoid granules are generally absent. The posterior or aflagellar end is in typical examples drawn out to a fine point, but not infrequently it is rounded off and quite obtuse. The centrosome stains very deeply, it is usually round, sometimes rod-shaped, and lies about 5·5 μ from the posterior extremity, but as the latter is a very variable factor, it may be as far as 11 μ. Immediately anteriorly to the centrosome is almost invariably an unstained area through which the attached part of the flagellum can be traced to the kinetonucleus.

The flagellum arises from or near the centrosome and runs forward obliquely through the vacuole to the surface of the body, forming the margin of the undulating membrane, and finally leaves the anterior end of the body as the free flagellum. The undulating membrane is thrown into a number of curves, generally five or six, and is consequently longer than the length of the trypanosome measured from the centrosome to the extreme anterior end.

The nucleus is situated in about the middle of the body. Usually it is in the form of a half circle with the convexity towards the concavity of the trypanosome. It is sometimes round, and measures about 4 μ in diameter.

With Giemsa's or Leishman's stain it appears as a somewhat dense collection of chromatin granules. When the stain has been more extracted the margins appear more deeply stained and consist of a number of granules with a central paler area. The free flagellum is about one eighth the length of the body of the parasite.

The Piriform type of the Trypanosome. This has a shorter but much broader shape, the average length of the body, without the flagellum, being about 20 μ and the width 8·8 μ. (Plate II, fig. 2.)

The anterior and posterior extremities are usually pointed, but in many the posterior end is rounded.

The protoplasm stains a faint blue colour, has a loose granular appearance, but possesses no evidence of myonemes. Irregularly shaped vacuoles may be seen in the protoplasm. The centrosome is either round or rod-shaped. The attached portion of the flagellum traverses the body obliquely two or three times in its course.

The free flagellum is longer than in the fusiform type, generally measuring about 7 μ, and is about one third the length of the body. The nucleus differs considerably from that found in the fusiform type. It is generally oval with its long axis at right angles to that of the parasite, and measures about 7 μ in length and 2·8 μ in breadth. It is almost invariably surrounded by a halo of unstained cytoplasm. In structure it is seen to consist of a peripheral layer of closely packed granules with a central fainter granular area.

As to the proportion of the two types of trypanosomes I found that of 126 parasites 56 were fusiform and 70 piriform in shape.

Subsequently I found the large fusiform trypanosomes in a blackbird on April 28, 1912, associated with *Halteridia*, and in a young blackbird on May 16, 1913, in conjunction with *Halteridia* and *Leucocytozoon*, but in both cases the trypanosomes were present in exceedingly small numbers, perhaps one being found in five or six films.

Small Trypanosomes of the Thrush.

In the blood of an apparently healthy thrush shot Aug. 29, 1911, I found the following parasites: 1. *Proteosoma*, very numerous. 2. *Trypanosoma*, small, fairly numerous, and two large forms. 3. *Leucocytozoa*, small infection, with forms of schizogony in the peripheral, heart and lung blood. 4. *Halteridia*, few, and 5th *Filaria*, embryos numerous.

As to an approximate number of trypanosomes: 21 films were made, 12 from the axillary vessels, eight from the blood of the heart, one from the lung. Over an area of a $\frac{3}{4}$ inch coverglass 91 trypanosomes were found in 18 films, three films were negative as regards trypanosomes, so that the average number found on a single film would be about $4\frac{1}{2}$. The largest number found on a single film was 26, this preparation was made from the heart about two hours after death. I should add that of the three slides which proved to be negative, two were fixed whilst moist with Schaudinn's sublimate mixture and stained with Heidenhain's iron-haematoxylin.

As these parasites belong to the small variety of trypanosome, and as they did not take on the basic blue colour of Giemsa or Leishman's

stain, it was necessary to examine each film carefully with an oil immersion, $\frac{1}{12}''$ objective and ocular 2 (approximately a magnification of 500 diameters), and even then it is quite possible that some were overlooked. Each slide was however searched very thoroughly, an average of three to four hours per film being spent.

The body of this trypanosome is somewhat fusiform, and is about $3\frac{1}{2}$ times as long as it is broad. The greatest width is at a position a little posterior to the middle, from whence it tapers off nearly equally at each end. The protoplasm stains a faint reddish-blue colour with Giemsa. It is for the most part granular and contains some vacuoles. In some specimens there are numerous chromatoid granules and this is especially noticeable in the trypanosomes found in films made from the heart and lungs. There were no indications of myonemes. (Plate II, fig. 3.)

The centrosome stains a deep crimson colour, and it is usually round or sometimes rod-shaped. Its position, about 2μ from the extreme posterior extremity, is fairly constant.

The flagellum seems to originate from the posterior end of the centrosome, and this is well seen when that structure is rod-shaped. It runs forwards obliquely crossing the body about twice and terminates as the free flagellum.

One very noticeable thing in practically all these trypanosomes is that the outer limitations of the undulating membrane stain a deep red colour, and running below it, and crossing it from time to time, is another line less deeply stained red. Whenever these two cross the body they seem to combine to form a single broad band, although it is not difficult even then to see which is the outermost or attached flagellum. Possibly these are mere folds in the periplast, but unlike somewhat similar structures described by Minchin in *T. lewisi*, they run more or less parallel.

The nucleus is situated about the middle of the body, and is somewhat round or oval in shape, with its long axis lying transversely to the long axis of the trypanosome. It measures on an average $3{\cdot}8\mu$ long and $2{\cdot}4\mu$ broad. Stained with Giemsa or Leishman's stain it appears to consist of a dense collection of chromatin granules, and sometimes shows some indication of division.

The free flagellum is about one half the length of the body. Out of the 91 trypanosomes two only belonged to the large type.

The Large Trypanosome of the Thrush. In addition to the two found in conjunction with the small variety, I met with examples of

the large fusiform trypanosomes, but only in very small numbers, in a very young thrush, July 28, 1912. Here they were associated with an enormous number of *Leucocytozoa*, and a few *Halteridia*.

These hardly differed in their general appearance and structure from the large fusiform trypanosomes which stain a deep blue colour, found in the blackbird. (Plate II, fig. 4.)

I have added a table of measurements of these trypanosomes. In the case of the trypanosome of the blackbird 40 parasites were drawn with a Zeiss camera lucida at an exact magnification of 2000 diameters, and in the case of the small trypanosome of the thrush 20 were drawn at 2000, and 20 at a magnification of 4000 diameters.

The post-nuclear region was measured from the tip of the posterior end to the centre of the centrosome, the internuclear region from the last named spot to the middle of the nucleus, and the pre-nuclear from the centre of the nucleus to the extreme anterior end. From the measurements given it would appear as if the large trypanosome of the thrush was considerably longer than that of the blackbird, but as a matter of fact the measurement of the latter was made upon a very large number of parasites, many of which showed blunt round posterior ends, whilst comparatively few measurements could be made of the large thrush trypanosomes as these were so infrequent. In other words the disparity in length depends somewhat on the variable length of the region from the centrosome to the posterior extremity, yet the large

Measurements of the trypanosomes found in blackbirds and thrushes.

		Total length	Pre-nuclear	Inter-nuclear	Post-nuclear	Greatest width	Free flagellum
Blackbird.							
A. Fusiform	Average	39·75 μ	18·0	11·6	5·3	7·2	4·6
	Maximum	48·0	22·0	12·5	6·5	8·0	7·0
	Minimum	33·0	16·0	10·0	2·5	7·0	4·5
Piriform	Average	26·8	8·25	8·8	2·8	8·8	6·9
	Maximum	30·0	9·5	9·5	5·0	8·5	7·0
	Minimum	22·5	5·5	7·5	2·0	9·5	4·5
Thrush.							
Small trypanosome	Average	28·92	8·65	8·27	1·9	5·6	10·0
	Maximum	31·5	10·0	8·0	2·0	5·0	11·5
	Minimum	23·5	5·5	7·0	1·0	8·0	10·0
Larger tryp.	Average of two	32·75	11·0	10·5	2·5	9·25	8·75
Large trypanosome	Average	52·0	24·5	9·75	10·25	7·75	7·5
	Maximum	59·0	28·0	9·0	12·0	6·0	10·0
	Minimum	46·0	24·0	9·5	4·5	9·0	8·0

trypanosome of the thrush is larger than that of the blackbird, whilst the two trypanosomes met with in the thrush containing the small trypanosomes are smaller, and many might regard the latter as the intermediate variety.

Trypanosomes of the Jay.

Six young jays (*Garrulus glandarius*) were shot and sent to me from Reading at the end of June, and by the time I examined the blood they had probably been dead about 18 to 20 hours. In films made from the blood of the heart I found large trypanosomes in five; the sixth jay was so mauled that it was impossible to make clean films and no prolonged search was made for them in this case. Three contained in addition *Leucocytozoa*, and in two *Halteridia* were present in moderate number.

The trypanosomes of the jay (Plate II, fig. 5) resemble the large fusiform variety met with in the thrush and blackbird. They stained a deep blue colour with Giemsa, but the nuclei, undulating membrane and the free flagella were very indifferently stained and in some cases invisible. The posterior extremity in the majority of cases was blunt and round, only a few specimens being found with the typical finely pointed end. Possibly the indifferent staining of the flagella and nuclei and the rounded blunt posterior extremity were evidences of post-mortem changes.

In many, myonemes were plainly visible.

These parasites were not very infrequent in one bird, as I found 17 trypanosomes in a large smear made from the heart blood.

The measurements of a typical trypanosome were as follows:

Total length	49·0 μ	Posterior end to middle of nucleus	22·0 μ
Length of body	43·0	Width of body	8·0
Length of flagellum	6·0	Nucleus length	4·5
Posterior end to middle of centrosome	10·5	Nucleus breadth	3·5

Centrosome diameter 1·0 μ.

Trypanosomes of Fish.

I have met with the following trypanosomes in fish:

T. granulosum in the eel (*Anguilla vulgaris*). This is by far the most commonly infected fish in this locality, and I do not remember to have examined an eel without finding trypanosomes. Often the parasites are

exceedingly numerous and all gradations in size are met with, so that it is often impossible to separate sharply the small from the large variety.

In the blood of one eel I saw a very large number of leucocytes containing round structures, and Prof. Laveran, to whom I sent a film, suggested that these might represent the remains of phagocyted red corpuscles.

T. percae in the perch (*Perca fluvialis*). Next to the eel this is probably the commonest fish containing parasites. I have found trypanosomes especially in the smaller fish. The size of the parasite varies considerably but the large forms with short flagella are more commonly met with than the small forms in which the flagella are longer.

T. remaki in the pike (*Esox lucius*). Two forms were found, var. *parva* and *magna*, which can be distinguished by the characters which Minchin (1909) has pointed out.

T. remaki parva, is clearer, has a few coarse granules, the undulating membrane is shallow and does not stand out sharply from the body, the flagellum is longer relatively and absolutely.

T. remaki magna, larger size, opaque protoplasm full of coarse granules staining reddish with Giemsa, the undulating membrane is very prominent. The kinetonucleus is slightly larger in *parva* than in *magna* (Minchin). I have only found these parasites in small numbers.

T. leucisci in the roach (*Leuciscus rutilus*) was very infrequent.

In the blood of sea-fish I have met the following trypanosomes:

T. solae in *Solea vulgaris*—the sole,
T. raiae in *Raia batis*—the skate,
T. scylii in *Scyllium catulus*—dogfish.

In many cases the trypanosomes were associated with *Haemogregarina*. I found haemogregarines in the sole, flounder, dogfish, skate and in the weaver (*Trachinus vipera*).

Haemogregarines or Leucocytogregarines
(Haemogregarina microti sp. n.?) *in Field Vole.*

In the blood of two field voles (*Microtus agrestis*), sent from Reading, June 24 and July 4, 1913, I found haemogregarines. I had previously examined from time to time several field voles caught in this locality but had not met with these parasites, whilst in the only

two specimens sent from Reading they were detected. Films were made from the peripheral and heart blood, lung, liver, spleen, and bone marrow. Parasites were found in the peripheral and heart blood and in smears from the lung and liver. They were never very frequent, but were more numerous in the lung, and were not detected in bone marrow.

The parasites are generally slightly curved, oval or sausage-shaped bodies with rounded ends, situated almost entirely in the mononuclear leucocytes. (Plate II, fig. 17.)

They measure on an average 9 to 10 μ in length and 3 to 4 μ in width. The protoplasm stains a reddish-blue colour and is slightly granular.

The nucleus, generally round or oblong in shape, is composed of a compact mass of chromatin and it is situated in the centre, or more generally, somewhat nearer one end of the parasite. It measures about 4·5 μ long and 2·75 μ in width. The breadth of the nucleus is not infrequently equal to that of the parasite, but sometimes the latter is much narrower.

This haemogregarine produces a marked karyolytic action on the nucleus of its host. It is rare to see a perfectly intact mononuclear leucocyte enclosing a parasite; for the most part the latter has invaded and split up the nucleus and the protoplasm evidently has become decolourised and finally invisible, and this condition prevails in good films in which the surrounding leucocytes are perfect. Very often the parasite lies between portions of the nucleus, and the outline of the leucocyte is quite lost. Sometimes the nucleus of the host completely surrounds and encloses the haemogregarine.

A few free haemogregarines were found, most frequently in the lung or heart blood, and these differ but slightly from the intra-leucocytic forms except that they may be a little narrower. The enclosed parasite is usually surrounded by a narrow unstained area, as if it were lying in a capsule.

Very numerous films of the blood and organs of both field voles were carefully examined for multiplication cysts, but in only one such smear, that made from the lung, was a cyst found. (Plate II, fig. 15.) This was oval in shape, measured 20 μ by 16 μ, and was surrounded by a narrow unstained halo. It stains a distinct blue colour with Giemsa, and contains a number of merozoites but these were evidently degenerated as their number, outline, or nucleus, could not be determined. The centre of the cyst consisted of granular débris also stained blue. Only

a very small number of haemogregarines were found in smears from the liver, and an occasional one from the spleen, but none were detected in films made from the bone marrow.

The haemogregarines were in one vole associated with Graham-Smith's bodies, *Grahamella*, both in the red corpuscles and in clusters free. In the other vole many of the large uninucleated leucocytes contained in their protoplasm a number of large spherical or oval granules which stained very deeply. Some of these granules measured as much as 3 μ in diameter.

Should this haemogregarine prove to be a new species, I would suggest that it be distinguished by the name *Haemogregarina microti* sp. n.

Haemogregarine in the Rat (Haemogregarina muris).

These were found in three common rats (*Mus decumanus*) all of which were dead, and two much mauled when examined. Haemogregarines were seen in films from the peripheral and heart blood, but not in those made from the liver, spleen or bone marrow. They were very scanty in the peripheral blood, but numerous in the blood taken from the thoracic and abdominal cavities, in which case they were contaminated with bacteria, probably from the gut.

The haemogregarines are somewhat crescentic, bean or sausage-shaped. The ends are round, generally equally so, but sometimes one is more pointed than the other. (Plate II, fig. 16.)

The body stains a bluish colour, and one end often a red colour whilst the nucleus stains a deep crimson colour with Giemsa. The protoplasm is granular and usually contains chromatoid granules which are most marked at one end and constitute a polar cap. Vacuoles are sometimes seen in the protoplasm.

The nucleus which consists of coarse granules of chromatin is frequently placed at one end, which may be pointed or somewhat hook-shaped, whilst the other end is packed with red granules. The nucleus in some cases shows a prolongation in the form of a very narrow line leading up to and terminating in a deeply stained granule.

The staining varies considerably; the greater number are stained a brilliant blue, some very deeply, others very faintly, whilst others are a pale red colour with numerous lightly stained granules scattered throughout the protoplasm. The nucleus, also, varies in its staining reaction; it may be deeply stained and appear almost black, or it may

be a very pale red colour. In the haemogregarines which are pale red in colour no nucleus can be detected.

Very commonly I met with two parasites lying together in a parallel way, or with their ends touching, and in these cases one is usually stained a deep blue colour, the other a pale blue colour.

The parasite shows considerable variation in its size but the average measurement of 20 specimens gave the following figures: length, 13 to 13·5 μ; breadth, 4 to 4·5 μ; the nucleus about 4 μ in both directions. I carefully examined several films from these rats but only once did I meet with a haemogregarine situated in the protoplasm of a leucocyte.

In one rat the haemogregarines were associated with *T. lewisi* and these were the longest and narrowest trypanosomes that I have met with in rats.

Haemogregarine in the Field Mouse (Haemogregarina sylvatici sp. n. ?).

In the blood of a long-tailed field mouse (*Mus sylvaticus*), caught in this neighbourhood, Apr. 7, 1912, I found haemogregarines which differed considerably in appearance from those previously described. Unfortunately only two films from the peripheral blood were kept. Associated with these haemogregarines was a small number of parasites situated in the red blood corpuscles which are probably of the nature of *Piroplasma* as will be evident when I describe them later on.

The haemogregarines were not very numerous, only about 20 being found in two films measuring about $\frac{3}{4}$ inch square. The parasites were only found free in the plasma; all the leucocytes in both preparations were carefully examined but none of the white cells harboured a parasite.

The haemogregarines are oval, kidney or bean-shaped bodies sometimes straight, more often slightly crescentic, in appearance. The ends are rounded, usually equally, but in some cases one extremity is much more pointed than the other.

They are not surrounded by a capsule. They measure on an average 15·5 μ in length by 6·1 μ in breadth. (Plate II, fig. 14.)

The protoplasm stains a deep blue colour with Giemsa and is very granular. It contains at one or both ends a large number of coarse chromatoid granules which stain deeply. In some there are fewer granules but these are much larger and even more deeply stained, and are often almost black in colour. Two haemogregarines were found in

which the granules are seen being discharged from the more pointed end of the parasite.

Sometimes towards one or both ends of the parasite are small areas in which the protoplasm is quite homogeneous and contains no granules. These patches are stained a very pale red colour with Giemsa, and seem to constitute what Dr Annie Porter describes as caps of chromatin. At other times similar patches are seen near the nucleus.

The nucleus varies in size, shape and position. Usually it lies nearer one end than the other, and may even be situated near the extreme end, at other times it is central in position.

It may be round, oval, square or triangular in shape and in one or two instances it constitutes a broad band running transversely across the parasite, from side to side, very much like the condition met with in the microgametes in tertian malaria fever.

In one case the nucleus, which was oval in shape, is seen to be clearly divided by lines into six or seven segments running parallel with the long axis of the parasite. This no doubt is evidence of the existence of myonemes.

The nucleus measures on an average 5·2 μ long and 5·6 μ broad.

The structure of the nucleus seems to be largely vesicular. Sometimes it consists of a number of chromatin granules closely packed together and at other times it is a dense deeply stained structure.

This haemogregarine seems closely allied to that described by Dr Annie Porter (1908), *Leucocytozoon musculi*. Should the parasite I have found prove to be a new species I would suggest the name *Haemogregarina sylvatici* sp. n.

The Haemamoebae of Bats—Pipistrelle.

I have met with two forms of intracorpuscular parasites in *Vesperugo pipistrellus*, a pigmented parasite, *Haemamoeba* or *Polychromophilus*, and an unpigmented parasite *Achromaticus vesperuginis*. The latter I have regarded as a *Piroplasma*.

The pigmented parasite is evidently not common, and I only met with it once out of 25 bats examined. It was found in the blood of a Pipistrelle caught in a room just outside the town on Oct. 18, 1912.

The following types were noticed in this specimen (Plate II, fig. 18):

1. Small signet ring-shaped parasites, measuring about 2 to 2·5 μ in diameter, situated sometimes near the centre but more frequently

towards the margin of the red corpuscle. The nucleus usually takes the form of a half or quarter segment of a circle. The protoplasm of the infected red cell shows no alteration in its staining reaction and no pigment is present.

These forms were very infrequently seen, not more than four or five being found in some 36 films which were very carefully examined. It is quite possible that these might not have been the young forms of the pigmented parasite but varieties of the unpigmented intracorpuscular parasite or *Achromaticus*.

2. Somewhat larger oval-shaped parasites measuring about 3 μ in diameter. The cytoplasm stains a pale blue and contains no pigment. The nucleus takes the form of a small segment of the oval or ring, or appears as a small oat-shaped mass situated at the margin of the parasite.

Not infrequently a very delicate line of nuclear matter extends from the nucleus right across the body and terminates in a spot lying near the margin which stains the same colour as the nucleus. I suppose by some this small body would be regarded as of the nature of a centrosome, but probably it might more correctly be considered as an extra-nuclear karyosome. This variety is also very seldom met with.

3. Still larger round or oval bodies measuring 4 μ in diameter, staining more deeply blue and with the nucleus situated towards the margin. One or more chromatinoid granules may be found in its interior, but there is no pigment, and the protoplasm of the red blood corpuscle shows no granules or polychromatophilous staining with Giemsa or Leishman's stain.

4. Parasites filling up $\frac{2}{3}$ or $\frac{3}{4}$ of the red cell, measuring about 5 μ, stained a deeper blue colour, with the nucleus situated towards the margin and from which occasionally a very fine line can be traced to a red stained granule lying in the protoplasm. The latter contains a small amount of pigment scattered throughout the protoplasm.

5. Parasites, circular or oval, showing no remains of the red cell, and containing much pigment. These are slightly larger than the surrounding red corpuscles and generally measure 6 to 7·5 μ in diameter.

Two types of these can be recognised, the macrogamete which stains a deep blue colour and in which the nucleus is compact, and the microgametocyte which stains either a faint blue colour and contains a diffuse large nucleus, or a distinct red colour in which it is difficult to determine precisely the boundary of the nuclear chromatin. Some of the latter are stained such a deep red colour, that they might very easily be

passed over as lymphocytes unless the particles of pigment were detected, but even these may be masked by the deep staining of the parasite.

Chromatinoid granules are occasionally seen in the macrogametes but they are much larger and more generally found in the microgametocytes.

Prolonged search was made in more than 36 films but no definite signs of segmentation were found, except the fact that in some few red stained parasites, four or five large chromatin granules were found, but even in these the pigment was scattered and not collected in the centre.

As to the frequency with which these haemamoebae were detected, I should think that in a perfectly spread film 15 to 20 parasites might be found, and almost invariably these were free and showed no evidence of the red corpuscle. My experience in this particular bat was that the smaller the parasite the rarer they were.

Films made from the bone marrow, spleen, and lung were examined and one or two parasites detected in the two latter organs but they differed in no way from those found in the peripheral and heart blood.

From the small amount of literature at my disposal, I find that Dionisi[1] has described three types of parasites found in bats:

1. *Polychromophilus melanipherus* from the blood of *Miniopterus schreibersii* which shows polychrome staining with Romanowsky.

2. *Polychromophilus murinus* from blood of *Vespertilo murinus* which also shows polychrome staining and contains pigment.

3. *Achromaticus vesperuginis* from the blood of *Vesperugo noctula* which contains no pigment and which according to Minchin appears from recent investigations of Yakimoff and others to be a true piroplasm.

The form I have found and described in the Pipistrelle apparently belongs to *Polychromophilus*, as it contains pigment, but the red blood corpuscles show no sign of polychromatophilous degeneration.

Achromaticus vesperuginis *in the Pipistrelle.*

Unpigmented parasites situated in the red blood corpuscles were found in 7 out of 20 Pipistrelles examined, but were not detected in the blood of three Noctules or two long-eared bats (*Plecotus auritus*). The degree of infection varied considerably. In two the infection was heavy and in one case as many as 15 to 20 parasites were found in the field of $\frac{1}{12}$ inch objective with compensating ocular 6. In this case they were

[1] See Lühe.

associated with a moderate infection of *T. vespertilionis*. In two others the parasites were moderately frequent and in one of these there was a very slight infection with trypanosomes. The remaining three bats contained very few parasites, and in one it required a prolonged search before a single parasite could be found. The infected Pipistrelles came from Reading.

The parasites are found almost entirely in the red corpuscles, exceedingly few free forms being met with.

They are usually round, oval or irregular in shape. The protoplasm stains a very faint blue colour whilst the chromatin is deeply stained with Giemsa.

The presence of the parasite does not seem to affect the staining reaction of the red corpuscles. In preparations stained with Leishman and Giemsa's stain the corpuscles show no sign of degeneration such as is seen in simple tertian and malignant tertian malaria, and in some forms of haemamoeba of the monkey.

The parasites usually lie somewhat out of the centre of the corpuscle, and there is no pigment at any stage in their development.

The commonest forms are round or oval with the nucleus occupying about $\frac{1}{3}$ or $\frac{1}{4}$ of the margin. Sometimes the parasite extends almost across the diameter of a red cell and then it assumes somewhat the shape of an egg, but true pear-shaped parasites are certainly uncommon. As the parasite becomes larger it occupies about $\frac{2}{3}$ of the red cell and the marginal chromatin shows signs of division into two, and a little later into four parts. Irregularly shaped amoeboid forms are common and in these the chromatin no longer occupies a marginal position. A red cell may contain three or four parasites but these are usually somewhat ill defined unless they are the immediate result of division.

It is somewhat difficult to determine what precisely is the method of division of these parasites, but it would appear as if the nucleus first divides into two, then four, nodules of chromatin, and these extend round the margin of the protoplasm until the whole parasite appears like a square having a nodule of chromatin at each corner. The protoplasm then seems to divide so that an appearance of a Maltese cross results, and the elements separate outwards, at first attached by a thin line of protoplasm at their apices. This breaks and one finds four small pear-shaped parasites arranged in the form of a cross. It is this method of division which suggests to my mind the probability of the relationship of the *Achromaticus* to one of the divisions of the piroplasms, possibly to the *Nuttallia*. (Plate IV, fig. 46.)

Gonder has stated that *Achromaticus* is intermediate between the malaria and piroplasm parasites and Ollwig and Manteufel[1] consider them closely related to the latter. Parasites showing division into four piriform parasites are very infrequently seen, but I have found them most clearly defined in films in which the parasites were not numerous.

Films from the internal organs, heart, lung, spleen, liver, kidney and bone marrow were made from all the infected bats, and a few parasites were found especially in the lung and heart blood.

Piroplasms in the Field Mouse—Nuttallia muris sp. n.?

In the blood of a field mouse caught in this neighbourhood, April 7, 1912, in addition to the haemogregarines already described, I found some small unpigmented bodies situated in the red blood corpuscles.

These were present only in very small numbers and were only to be found by careful examination. One of the most noticeable features about them was not so much their small size as their marked transparency, and it is quite easy to go over the whole slide with an immersion objective and low eyepiece and miss them entirely. In fact when I have found a parasite and noted the exact position by the reading on the mechanical stage, and have further marked the spot with a small diamond ring on the coverglass, I have not infrequently experienced the greatest difficulty in detecting the same parasite again.

These intracorpuscular bodies vary in their size and shape, but by far the commonest is a round or oval shaped structure, which occupies a position just outside the centre of the corpuscle. Sometimes they are entirely on the margin and may actually project beyond the outline of the red cell. (Plate II, fig. 20.)

The central part is stained very faintly a blue colour, often it is almost colourless. The margins are more deeply stained, in part a chromatin red and in part a blue tint.

The smallest and presumably therefore the youngest forms are somewhat elongated oval or round, the largest are round or oval, rarely irregular in outline. The smallest parasites generally show a red stained spot at one end, and sometimes there are two such nuclei at opposite sides of the ring.

The larger oval forms appear to contain more chromatin, which extends $\frac{1}{2}$ to $\frac{3}{4}$ round the margin. Vacuoles are occasionally but rarely

[1] See Prowazek (1912).

seen. Very usually an extremely fine line, not easily seen, extends from the nucleus across the centre to the other side of the organism, whilst at other times a strand of nuclear chromatin, much thicker than the above mentioned line, extends $\frac{1}{4}$ to $\frac{1}{2}$ the distance across the body.

Distinct pear-shaped parasites were extremely rare, but one example was seen in which the narrow end was drawn out to a fine point. A few examples of dividing parasites were met with, shaped somewhat like a loaf of bread, but these were round or oval and not at all pear-shaped. Usually the dividing forms appeared like a loaf of bread or figure 8, or in some instances like a trefoil leaf.

Irregular or amoeboid forms were very infrequent. One of these showed a fine line extending from the nucleus into the protoplasm of the red cell and terminating in a deeply stained red dot. In no case did I meet with two parasites in one red cell. No definite free parasite was seen, but I can quite imagine that these minute structures might easily be overlooked.

The average diameter of the red corpuscle in the field mouse is about $6\,\mu$, and the small parasites measure about $1\,\mu$ by $2{\cdot}3\,\mu$, some probably less. The average size of the commonest parasite, which is round or oval, is rather less than $3\,\mu$.

The red corpuscles in which the parasites are situated show no appreciable alteration in size or staining reaction and the parasites have no pigment.

As I thought these unpigmented ring-like parasites might represent the early stage of a larger intracorpuscular haemamoeba, very careful search was made for such but with entirely negative results.

It would seem therefore as if these intracorpuscular parasites belong to the piroplasms used in its fullest sense.

Recently I find that França (1912) has described somewhat similar parasites in *Microtus incertus* which he has named *Smithia microti*, but unfortunately I have been unable to see his original article. If the parasite found in the field mouse be a new species I should rather incline to the opinion that it belongs to the *Nuttallia* and would suggest the name *Nuttallia muris* sp. n.

Piroplasms in the Water Vole—Nuttallia microti sp. n.?

I found in the blood of a water vole, *Microtus amphibius*, caught in this neighbourhood, April 15, 1912 (but not in the same district as the field mouse), similar unpigmented intracorpuscular bodies closely resembling those of the field mouse.

The parasites were exceedingly infrequent, and even in my preparations I should not expect to find one in less than 15 to 20 minutes careful search.

Most commonly they are situated somewhat outside the centre of the red corpuscle, and at times they are quite on the margin. (Plate II, fig. 19.)

By far the commonest is a round or oval-shaped body, very faintly stained blue in the centre and surrounded by a denser ring, half of which is stained red, the other half blue. There is no pigment. Pear-shaped parasites were exceedingly rare, but dividing forms were sometimes met with.

The nucleus is placed on the margin of the parasite and extends as a cap or segment $\frac{1}{6}$ to $\frac{1}{2}$ way round. Sometimes the chromatin is split up into two, three or four little masses which are nearly always placed on the margin. Minute points of chromatin, situated a little distance from the nucleus, could in some cases be detected, representing the centrosome of some writers, more probably extra-nuclear karyosomes. In some of the parasites on very careful examination a distinct fine line could be seen extending from the nucleus, across the body and terminating in a red stained spot on the other margin of the ring, just as was the case with those found in the field mouse.

I found forms resembling a loaf of bread or figure of 8, as if budding had taken place. A few divided mesially, in the long axis, into two, each of which had a nucleus, whilst in others the two minute parasites were separating and were shaped like the letter **V**.

I found two specimens in which division into four had taken place, in one the four young parasites were attached to one another, in the other four minute pear or oval-shaped parasites were arranged in the form of a cross. (Plate II, fig. 19 and Plate IV, fig. 45.)

Generally speaking these parasites closely resemble those found in the field mouse, but they were on the whole a little smaller.

The average diameter of the red corpuscle of the water vole I found to be about 5·9 μ, and the average size of the enclosed parasite varies from 1 to 2 μ, none apparently exceeding 2·5 μ in diameter.

If this represents a new species I would suggest the name *Nuttallia microti* sp. n.?

Piroplasma divergens in Cow.

I found these piroplasms in three cases of Redwater Fever in cows locally. The parasite is more generally known as *Piroplasma bigeminum* or *P. bovis*, but as Prof. Nuttall pointed out to me the species met with in England is *P. divergens*.

The number of red corpuscles containing parasites varies enormously, in one case only about 2 % were infected, whilst in another 23 % of the corpuscles contained one or more parasites.

In one case the piroplasms were associated with the presence of the large trypanosome which I have previously described.

The infected corpuscle most commonly contained a single round parasite which is usually situated towards the margin. Double pear-shaped parasites were fairly numerous but in no case did I meet with two double piriform parasites in one corpuscle. A comparatively small percentage showed the method of multiplication described by Nuttall (1908) in which "the peculiar piriform parasites develop through a process resembling budding from a large, rounded, or slightly amoeboid parasite, the whole of whose protoplasm flows into the 'buds' and gives rise as a rule to two piriform parasites without a residual body being left" (Nuttall). Free piriform parasites were extremely infrequent.

The Halteridia of Birds.

Halteridia were found in numerous birds, especially the thrush, blackbird, starling, finch, jay, etc. Generally the infection was slight but in very young birds, particularly the thrush and blackbird, parasites were exceedingly numerous. They were nearly always found in association with other parasites, *e.g.* leucocytozoa, filaria or rarely trypanosomes. All stages were seen and some excellent permanent preparations were obtained of flagellating bodies and ookinetes. (Plate III, figs. 24, 25, 26, 28 and 29.)

I was struck with the diversity of appearance of halteridia met with in the same and in different species of birds. They showed differences in their size, shape, nature of the protoplasm, nucleus, and in the quantity and distribution of the melanin pigment, and these factors were apparently independent of the age and sex of the parasite or the method of staining adopted.

During an examination of the blood of birds extending over more than fifteen years I have never met with the slightest indications of the transformation of the halteridium into a trypanosome.

The Leucocytozoa of Birds.

In my experience *Leucocytozoa* are by far the commonest parasites found in the blood of birds. I have seen them in the thrush, blackbird, jay, pigeon, starling, moorhen and many others; in fact I should expect to find them in almost any of the common birds, at any time of the year, often however in small numbers.

In the blood of the blackbird and thrush, especially in young birds, I have met with an enormous infection, more than 20 leucocytozoa being seen in the field of $\frac{1}{12}$ objective with a low ocular, in fact at the first glance at the preparation with a lower power one might think we were dealing with a case of leukaemia. In the birds I have examined, only the round or spherical forms were met with. I have never yet seen the spindle-shaped leucocytozoon. It may be argued that the forms I have met with have been the spindle-shaped specimens which have become rounded off after the blood was taken from the body. I am convinced that this has not been the case (except perhaps in a few cases in which the bird had been dead some time) as films were made and fixed at once by osmic acid vapour, or a fresh drop of blood was covered with a coverglass and examined immediately, and in no case has there been any approach to the appearance of a spindle-shaped body. That I should not have met with the spindle-shaped form is the more remarkable as I detected and made drawings of leucocytozoa as far back as 1904, but I then had no idea of their nature. (Plate III, figs. 27, 30 to 44.)

Generally speaking the blood of a bird which contains these parasites shows only one stage of the leucocytozoa, usually the adult, at one time, and it is not common, except in the case of very young birds, to meet with all stages from the youngest to the fully grown parasite.

It is difficult to dogmatise as to the nature of the host cell, generally it seems to be a leucocyte or an immature red cell. I am not convinced that leucocytozoa never contain pigment.

It is common to find two or three young parasites in one host, and I have seen, in the blood of a young blackbird which had a very severe infection, five small round or oval parasites in one immature cell. In the adult stage two parasites, of the same or opposite sex, may not infrequently inhabit the same cell.

The adult female *Leucocytozoon* shows a compact deeply stained nucleus in or near which is seen a granule which stains more deeply with Giemsa. In the adult male the nucleus is large, oval, and diffuse,

consisting of granules or threads which stain a paler colour than that of the female, and near the margins two or three grains may sometimes be found which stain more deeply with Giemsa. These structures are evidently those which were regarded by Schaudinn and others as centrosomes, but which Woodcock considers to be karyosomatic elements.

The average measurement of the leucocytozoa is about 10 μ in diameter—whilst the host cell is about 15 μ—but they may be considerably larger than this.

The formation of flagellating bodies and ookinetes was seen in fresh preparations and these bodies were also found in stained films. (Plate III, figs. 30 and 31.)

The internal organs of some of the infected blackbirds and thrushes, *viz.* the spleen, bone marrow, lung, etc. were examined, but I have not been able to detect any figures of schizogony of the leucocytozoa resembling those which Fantham (1910) has described in the spleen in the case of *Leucocytozoon lovati*, but in one thrush I found peculiar granular bodies in the blood which I think may represent forms of multiplication in the leucocytozoon of that bird.

Schizogony of Leucocytozoon of the Thrush.

In the blood of the thrush, which has been mentioned before, which was shot on August 29, 1911, locally and which contained a mixed infection of: *Proteosoma*, large infection; *Halteridia*, very few; *Leucocytozoa*, few; *Trypanosoma*, small and large; and *Filaria*, numerous embryos; I found a few large bodies containing large red nodules. These bodies were found in (*a*) the peripheral blood taken from the axillary vessels, (*b*) the blood from the heart, and (*c*) in smears from the lung. The films from the axillary vessels were made immediately after death, those from the heart and lungs some two hours subsequently.

As to the frequency of these bodies: in all 21 films were made from this bird, one from the lung contained 17; 8 from the blood of heart contained 11; and 12 films from the axillary vessels contained 4 of these granular bodies. It will be seen that more than half of these peculiar bodies were found in the one film made from the lung, whilst only four actual specimens, and these are not so striking in aspect, from the numerous films made from the peripheral vessels.

These structures are round, oval, or somewhat irregularly shaped bodies staining a blue colour with Giemsa, and contain a number of red stained bodies. (Plate III, figs. 33 to 44.)

The average measurement of 20 specimens was 17·2 μ by 14·2 μ; the largest, oval in shape, was 23·3 μ by 18·3 μ and the smallest, 8·3 μ by 6·6 μ.

Some of these bodies, which stain a deep blue colour, contain only a few nuclear masses, whilst others are so densely crowded with smaller nuclear masses, that none of the background is seen, and the whole parasite consists of a dense mass of red granules packed closely together giving the general impression of a strawberry. I met with one such densely red stained body in the film made from the lung. It was almost perfectly round and measured 19 μ in diameter. (Plate III, fig. 35.)

Generally speaking the more numerous the nuclear masses the smaller they are, whilst in those bodies which contain few nuclear bodies the latter are considerably larger, and may measure 3 μ in diameter.

The size and number of the nuclear masses vary considerably. When very numerous they are probably about 1 μ in diameter, but more usually they are about 2 μ. They are round, oval or more usually irregular in outline and show very little structure when examined with the highest powers. The smallest number seen in one cell was four, the usual number about 15 to 25, whilst in some they were quite beyond count. Vacuoles are commonly found in all these parasites except those which contain very numerous nuclear bodies, and occasionally one can see a few of the latter lying free in the plasma as if they had recently escaped.

I first met with these large and conspicuous bodies, remarkable alike for their size and brilliant colouring, in a film from the heart blood, and it was difficult to say what they represented. In general appearance they closely resembled a large free female leucocytozoon, so commonly met with in some films which contain many of these parasites, but differed from this by the presence of so many nuclei. I could however find no such bodies in any of the leucocytes in films from the peripheral or heart blood, only the free forms could be seen. I subsequently examined carefully the film made from the lung and here found a very few similar bodies inside the leucocyte.

I found in the lung film, which by the by consisted apparently only of capillary blood from this organ, a few large leucocytes containing a round or oval body in their protoplasm which had a large nucleus deeply stained red. (Plate IV, fig. 39.) These might have been ordinary young leucocytozoa, but they differed from these only in the relatively large nucleus they possessed. Next I met with a few leucocytes containing one or two leucocytozoa almost mature in size, which differed from the

macro- or microgamete stage of an ordinary leucocytozoon by the presence of numerous red bodies, obviously chromatin, and also by the presence of oval or circular vacuoles representing apparently places previously occupied by a nuclear body. (Plate III, figs. 37, 39, 40, 41, 42.)

Lastly I came across a few multi-nucleated leucocytozoa around which were the remnants of the nucleus of the host cell.

One could therefore, I think almost definitely, say that the large blue free bodies with numerous nuclei met with in very small numbers in the peripheral blood, more numerous in the heart blood and considerably more frequently in the blood from the lung, were originally occupants of the protoplasm of the leucocytes, just as the leucocytozoa are, and there can be little doubt that they represent a multiplying phase of the leucocytozoon of the thrush which probably takes place in the lung.

It is rather remarkable that although I have examined, during the last ten years, the blood of a very large number of thrushes and blackbirds (I have especially confined my attention to these as they are easily procured) I have never met with analogous structures in the blood from the peripheral vessels or heart. I have unfortunately not examined the blood from the lungs, spleen, or bone marrow, nearly as frequently.

Proteosoma of Birds.

I regard this as an extremely rare infection of birds in this locality, and have only met with these intracorpuscular parasites in three thrushes and in one blackbird. In two of the thrushes the *Proteosoma* were exceedingly scanty and they were associated with *Leucocytozoa* and micro-filariae. In one thrush they were very numerous and were found associated with halteridia, leucocytozoa, trypanosomes and micro-filariae. In the blackbird the *Proteosoma* were extremely infrequent and were associated with *Halteridia*.

I have been able to follow in the thrush all stages in the development of this parasite, but did not manage to make permanent preparations of the flagellating forms.

Spirochaetes in the Bat. Spirochaeta vesperuginis sp. n. ♀

The blood of 20 Pipistrelles, the common small bat, 3 Noctules or large short-eared bats and 2 large long-eared bats was examined and I found spirochaetes in one Pipistrelle (*Vesperugo pipistrellus*), and in one Noctule (*Vesperugo noctula*), both of which were sent from Reading.

The Spirochaete in the Pipistrelle.

The spirochaetes are present in very small numbers, on an average not more than two or three being found in a film measuring 1 by $\frac{7}{8}$ inch.

Films were made from the peripheral blood, heart, lung, spleen, liver and kidney, but spirochaetes were only found in the peripheral and heart blood and in that from the lung.

They were associated with a very large number of the unpigmented intracorpuscular parasites (*Achromaticus*).

Measurements. The average measurement of 100 spirochaetes was 15·37 μ long with 7·5 turns or spirals. The shortest specimen found was 10 μ in length with 5 turns, the longest 23·3 μ long with 12 turns. The filament of the spirochaete measured about 0·2 to 0·3 μ in thickness: the distance of the summit of one spiral to that of the next spiral was about 2 μ, whilst the depth of the turns measured from the level of the summit of one to the level of the summit of another spiral varied from about 0·5 to 1·0 μ. These latter measurements were taken from photo-micrographs. (Plate II, fig. 21 and Plate IV, fig. 47.)

The longitudinal axis of the spirochaetes was generally straight or slightly curved, a few were bent at right angles. The spirals are for the most part very regular, being somewhat deeper in the centre and gradually diminishing towards the extremities. The ends of the spirochaetes are drawn out to a fine point, but I could in no case, even after prolonged examination, detect the presence of flagella or terminal filaments.

Almost invariably long specimens show signs of transverse division, the central wave or turn becoming flattened out, thinner, and more faintly stained, whilst in other cases a distinct break in the continuity of the filament was seen.

During the examination of 103 spirochaetes I saw no indication of longitudinal division taking place, and in only one was the filament recurved.

This spirochaete constantly stains a reddish colour with Giemsa, never a blue tint.

The parasites met with in the blood from the heart and lung showed practically no difference from those found in the peripheral circulation, except that the spirals in the former were a little deeper and more pronounced than in the latter.

The Spirochaete in the Noctule.

In this bat there were even fewer spirochaetes than in the Pipistrelle and the blood contained no other parasites except an embryo filaria which I found in one slide.

Films were made from the peripheral blood, heart, lung, spleen, kidney and liver, but spirochaetes were only found in those from the peripheral vessels and heart, and even here they were present in extremely small numbers.

The measurement of 48 specimens, all that could be found in a large number of preparations, showed them to be 15·9 μ in length with 8·1 spirals. The longest which might be dividing was 25 μ in length with 12 turns, the shortest 10 μ with 6 turns.

The spirochaetes were straight or slightly curved with regular shallow spirals which were somewhat deeper and more pronounced in the centre and which gradually diminished towards the extremities. The ends were sharply pointed but no flagella could be detected.

They closely resembled those found in the Pipistrelle, and also stained red with Giemsa. (Plate II, fig. 22 and Plate IV, fig. 48.)

It is interesting to note that both in the Pipistrelle and Noctule it was practically useless to search for the spirochaetes in any position of the blood film except at the very commencement of the smear. During an examination of more than 100 films I never once detected a spirochaete at the end of the smear, probably only once or twice in the middle of the smear, and in all other cases they were found in the first $\frac{1}{4}$ or $\frac{1}{3}$ of the film. This peculiarity applies also to the position of *T. vespertilionis*.

Method of examination suitable for detection of these and possibly other spirochaetes. I discovered the first spirochaete whilst I was examining a blood film of the Pipistrelle for dividing forms of *Achromaticus* which were very numerous in that particular bat. The preparation was stained, and mounted in parolein and I was using the $\frac{1}{12}''$ objective. I may add that it is exceedingly easy to pass over one of these fine spirochaetes without detecting it, even with a magnification of 600 or more, and the search for them in many films is well nigh hopeless. After I had found a spirochaete, I adopted a method for their detection which I have long used for finding *S. pallida*, and which I have not seen described yet. The method depends on the principle: 1st, that structures which are stained with a fluorescent dye, *e.g.* eosin, fuchsin, fluorescin, etc., when examined

on a dark ground stand out prominently, whilst blue stained structures are very inconspicuous; 2nd, that if the medium in which the red stained organism lie be of very low refractive index they are exceedingly conspicuous and can be detected with a comparatively low power; but if mounted in balsam, cedar oil, parolein or water they are only seen with difficulty.

Films are stained with Giemsa, Leishman, etc. in the ordinary way if they are going to be kept and subsequently examined with an immersion lens; if not, with eosin, fuchsin or even red ink for a few minutes. Wash, dry, but do not mount. Examine dry, without any mounting medium at all, with dark ground illumination, using dry lens and moderate ocular. I use $\frac{1}{3}$ apo. with comp. ocular 6 or 12 or No. 18 for detection of *S. pallida* and dark ground illumination obtained by means of a stop used with an achromatic condenser, as with the oil immersion condensers the oil is troublesome if one has to examine a large area. I do not put a coverglass on the preparation, but lengthen the tube of the microscope about 3 to 4 inches to get perfect definition.

By this means I can detect 50 spirochaetes in less time than I could find one with $\frac{1}{12}$ oil immersion used with ordinary illumination. I mark the position of the spirochaete by means of a diamond object marker, and log its position on the mechanical stage, and subsequently mount the preparation in parolein, and examine at leisure with the $\frac{1}{12}$ oil immersion lens.

The spirochaetes found in the pipistrelle and in the noctule were very similar in size, number of spirals and staining reaction and probably belong to the same species.

Nicolle and Comte have found that bats in Northern Africa suffer from fever due to the presence of spirochaetes which they have named *S. vespertilionis*. I have not been able to find a description of these, and it is quite possible that those I have found in bats in England may be identical. If however they should prove to be a new species I would suggest the name of *Spirochaeta vesperuginis*, sp. n.

I examined some of the ectoparasites, especially ticks, found on some of the bats but with entirely negative results.

Grahamella or Graham-Smith's Bodies.

I have found these in the blood of the mole, field mouse, field vole, water vole and in very young rats. They are almost always to be found in the mole sometimes associated with *T. talpae* and sometimes with *Elleipsisoma thomsoni*.

In four or five young rats killed in a nest near the river, which were about seven days old, as the eyes were not opened, these bodies were very numerous. I am not quite certain whether these were the young of the ordinary rat or of the water vole, but judging from the shape of the head, I think they were the former.

The blood showed a large number of polychromatic, bluish stained corpuscles, and also a large number of Graham-Smith's bodies in the corpuscles and free in the plasma. The number of infected corpuscles varied, but generally there were three in every field of the $\frac{1}{12}$ inch objective.

These bodies differed in number and in size. In some corpuscles they were so densely packed together that the whole cell stained a lilac colour, and the background of the red blood was quite obscured. In others, especially in some of the large pale corpuscles, there were very few, probably not more than five or six in number. (Plate IV, fig. 50.)

The intracorpuscular and the free bodies consisted of irregular rods, sometimes thicker at one end than another, club-shaped or beaded, and resembled very much *Bacillus diphtheriae*. They do not stain a pale blue colour like the basophile granulations so commonly seen in anaemic conditions of the blood, but a peculiar lilac colour.

It seemed as if the infected corpuscle became enlarged, and much paler, and eventually disappeared leaving a group free in a cluster. Many of the free groups or clusters were about the size and shape of a corpuscle, and in a very few cases a faint outline of the original red cell could be detected. Others were scattered loosely in the field, and these as well as the intra-cellular forms stained in varying degrees of intensity, and not uncommonly one or two of the bodies appeared much larger, irregularly round or oval in shape, and stained a deeper colour than the others. Some of the groups no longer consisted of rods, but had degenerated into mere granules.

Many of the Graham-Smith bodies measure 1 to 1·5 μ long and 0·2 to 0·3 μ in width.

Peculiar Bodies in the Blood of the Mole.

In the blood of one or two moles I found bodies which are not normal elements of the blood and yet which I could not classify. These occur only in small numbers, not more than six or nine could be found in a film. (Plate II, fig. 23.)

They are usually oval, sometimes round, in shape and measure about 12 to 16 μ in length and 5 to 8 μ in breadth. The protoplasm stains a blue colour with Giemsa. They contain a varying number of nuclei. In some there are two or three large round nuclei and four to six small round granules, in others there are five or six large round nuclei and two or three small red stained granules, whilst in a few there is only one large pale nucleus. To my mind a few look somewhat like leucocytes which are undergoing pycnotic degeneration, whilst others suggest the appearance of haemogregarines.

I have as far as I can remember only met with them in the mole, and in the blood of rat which contained definite haemogregarines.

Elleipsisoma thomsoni.

These parasites, discovered by Thomson and named by França, I occasionally found in the blood of the mole. In some cases they were associated with *Grahamella* and in others with *T. talpae*, often with neither. The adult bodies are round or oval measuring about 10 μ by 5 μ.

The protoplasm in my experience stains a very pale blue, or even a reddish, colour with Giemsa, and in some cases contains granules scattered irregularly throughout its substance. The nucleus varies considerably in size and shape. In some it is diffuse, stains lightly, and occupies about one half of the whole parasite; in other cases it is compact, more deeply stained and measures about 3 to 4 μ in diameter. In all cases the nucleus is situated at the margin of the body. The parasite contains no pigment.

I have sometimes found a few free vermiform bodies with small oval nuclei.

Micro-filariae.

Embryo Filaria in the Mole.

In the peripheral blood of a mole I found one embryo *Filaria* having the following characters.

It was almost straight, earth-worm like in shape, with the anterior end rounded. For nearly its whole length it had the same dimensions, and ended somewhat abruptly in a pointed tail. I do not think it possessed a sheath, although at the anterior and posterior ends it was devoid of nuclei.

The anterior half consists of closely packed nuclei which terminate about 6 μ from the end, in the posterior half the nuclei are arranged loosely in a sort of adenoid reticulum which also ends about 6 μ from the extreme end.

It measured about 270 μ long and 16·5 μ in width.

Embryo Filaria in the Bat.

In the peripheral blood of a noctule one micro-filaria, associated with spirochaetes, was found. The parasite, which was enclosed in a sheath a little longer than the embryo filaria, was full of closely packed large nuclei. The oral extremity is blunt, and from thence the parasite at first diminishes and then, at a distance of about 10 μ, expands in width for a short distance. Afterwards it gradually gets thinner and ends in a sharp tail, which is slightly coiled up in the sheath.

The measurement of this micro-filaria was: 110 μ long, 6 μ in width near the anterior end, and 5 μ wide in the middle. The width of the sheath was about 8·5 to 9 μ and its length slightly less than that of the contained parasite.

Embryo Filariae in Birds.

Embryo filariae are extremely commonly met with in the blood of blackbirds and thrushes, and I should think that next to the *Leucocytozoa* they are the commonest blood parasite met with in these birds. They vary enormously in their length, some measuring about 140 to 150 μ and 5 μ in width, others reaching more than 400 μ with a transverse measurement of 8 μ.

These parasites are often associated with *Halteridia*, *Leucocytozoa* and sometimes *Trypanosoma*. The blood not infrequently shows an increase in the percentage of the eosinophile cells.

Protomonadina.

Whilst examining the blood taken from the heart of the common lizard (*Lacerta* sp.) which had been dead about two hours I met with several examples of flagellate protozoa.

These were usually round, sometimes oval, rarely pear-shaped. They measure about 8 to 12 μ in diameter, and stain a pale blue colour with Giemsa. The protoplasm is granular, and towards the margin of the cell is situated the nucleus which stains a deep red colour. From a point near, but not touching the nucleus, one, usually two, long flagella,

arise, which measure about 40 μ in length. As a rule there were two flagella, one being thicker and more deeply stained than the other. In some cases they run in the same direction, in others they proceed in opposite directions. Some specimens had two nuclei either on the same or on opposite sides of the organism, and from each proceeded two flagella of unequal thickness.

These organisms I take to be examples of *Monas* or *Bodo*, and they are I believe common inhabitants of the digestive tract of various animals, especially *Lacerta*. It is possible that the examples I met with were accidental, having come from the intestinal tract. If this were the case I should have expected to have seen various other signs of intestinal contamination, but the films were very clear, and Plimmer has found similar flagellates in the blood of reptiles. I afterwards learnt that the lizard in which I found these structures, had been kept in captivity for a few days before it was killed. Minchin (1912) states that " in some cases an intestinal parasite may pass from the intestines into the blood or lymph under certain pathological conditions of the host."

Leucocyte Inclusions.

In the blood of the field vole I have not infrequently met with large granules in the protoplasm of the large uninucleated leucocytes.

These granules stain a deep red, almost black, colour with Giemsa. They vary considerably in size. In leucocytes where they are very numerous they are not much larger than eosinophile granules, whilst in others, in which there may be few, they measure as much as 2·5 to 3 μ in diameter. (Plate IV, fig. 49.)

They are generally perfectly round, sometimes oval, and rarely elongated.

In the blood of one field vole 40 % of all the leucocytes contained these granules. They were present also, in smaller numbers, in one of the field voles which showed haemogregarines.

I have also found them in other animals, especially in some of the large mononuclear leucocytes of a cow suffering from Redwater Fever.

I imagine that these are not protozoal, but probably of the nature of secretion products.

Toxoplasms and Haemogregarines of the Squirrel.

Toxoplasma sciuri et Haemogregarina sciuri sp. nov.

Two squirrels purchased in London were kept in an aviary with two American grey squirrels. The English squirrels died and their bodies were taken to Mr Rohu a taxidermist (of Bournemouth) to be mounted. He kindly brought me the heart, lungs and liver of one of the squirrels in order that I might examine the blood. I would here express my thanks to Mr Rohu for these and several other specimens that he has sent me.

In the heart blood, liver, and lungs I found both *Toxoplasmae* and *Haemogregarinae.*

As far as I know, quoting from Miss H. L. M. Pixell (1913) *Toxoplasmae* have only been found in

1. The Gondi, *Tox. gondii,* in Tunisia by Nicolle and Manceaux.
2. Rabbit, *Tox. cuniculi,* (Carini) in Brazil.
3. Dogs, *Tox. canis,* (Mello) in Italy and Brazil.
4. Moles, *Tox. talpae,* (Prowazek) in Japan.
5. Pigeons, *Tox. columbae,* (Yakimoff and Kohl-Yakimoff) Brazil.

Toxoplasma of the Squirrel.

Parasites were very numerous in the liver, less numerous in the lung, and very scanty in the heart blood, although they were first detected in the latter. They were found free and in the protoplasm of the leucocytes and endothelial cells.

Free Toxoplasma. The free parasites occur as small crescentic oval or rarely round shaped bodies. The crescentic shaped free bodies are in appearance somewhat intermediate between a malarial crescent and a Leishman-Donovan body but they are much smaller than the former. (Plate V.) Usually one end of the crescent is more pointed than the other, sometimes however the ends are symmetrical. The crescentic shaped forms are probably the most numerous whilst the round form is quite infrequent. Commonly these small bodies are single, but double forms, evidently dividing parasites, are also very common.

They are also found in fours, just separated or closely bound together. Circular agglomerations of 6, 8, 12 or even 20 bodies are not uncommonly met with. (Plate V, figs. 17, 19, 22.) Occasionally a compact group of 50 to 60 parasites is seen. (Plate V, fig. 23.) In

some the individual members of the group are very clearly defined, whilst in others they are more or less fused together.

The average measurement of the free *Toxoplasma* is about 5 to 6 μ in length and 2 to 3 μ broad. Compact groups of four parasites, usually circular in outline, measure about 7 μ in diameter, whilst not infrequently, especially in the liver, more rarely in the lung, groups of 50 to 60 elements measuring 25 μ or more in diameter, were met with.

The protoplasm is alveolar and stains a bluish, less frequently, a bluish-red colour with Giemsa. It often contains chromatoid granules but vacuoles were seldom seen.

The nucleus, stained a brilliant-red with Giemsa, is generally situated about the middle of the body, sometimes however it is nearer one end than the other. It consists of an irregular mass of chromatin granules.

No definite centrosome can be made out, although the nucleus not infrequently stains more deeply at one point at the margin.

Near the nucleus one or two deeply stained granules are often seen, whilst in some of the parasites a fine red stained line can be seen extending from the nucleus to a granule situated at one pole of the body. (Plate V, figs. 13 and 14.)

Intracellular Toxoplasma. The *Toxoplasmae* are found in the protoplasm of the mononuclear and occasionally polynuclear leucocytes, and in the large endothelial cells. (Plate V, figs. 20 and 21.) Occasionally a single parasite or its remains could be detected in a liver cell.

The number of parasites enclosed in the host cell is very variable, sometimes there are only one or two, at other times the protoplasm is crowded with them. They may be different throughout the cytoplasm or grouped together in masses.

On one occasion I found a leucocyte containing a *Haemogregarine* and also a *Toxoplasma.*

In many cases it is difficult to determine the exact outline of the individual parasites and at times the nuclei and granular debris are all that remains.

This may be explained (α) by post-mortem changes which have taken place as the organs were not examined till 24 hours after death, (β) by phagocytic action of the host cell, or (γ) by some secretion of the parasite itself.

Although round masses of 20 to 30 or even 60 parasites were not infrequently found, and which to some extent resembled cysts, yet there was no evidence of a capsule (Laveran and Marullaz, 1913, speak

of these in the gondi as pseudo-cysts). This is in marked contrast to the true multiplication cysts of *Haemogregarines* also present in this case. (Plate V, fig. 12.)

On two occasions a crescentic shaped *Toxoplasma* was found lying on or in a red blood corpuscle. The focus of the parasite and of the red cell was precisely the same, but as only two perfect examples were found in numerous films it is probable that the parasites were merely lying accidentally on the surface of the corpuscle.

As to the distribution of the parasites. In the blood taken from the heart only few examples of free forms or an occasional leucocyte containing one or two parasites were seen, and even these might have been accidental. In smears from the lung free forms both single, double, in groups of four or more elements as well as the intracellular forms were much more numerous, whilst in smears made from the liver all forms were exceedingly numerous.

Multiplication takes place by binary fission. The toxoplasm becomes oval or spherical, the nucleus separates into two, and the protoplasm divides longitudinally, very rarely transversely.

Haemogregarine of the Squirrel.

I found, associated with the presence of *Toxoplasma*, haemogregarines both free or enclosed within the protoplasm of the mononuclear leucocytes.

These were most numerous in films made from the lungs, less numerous in the blood taken from the heart, and rare in liver smears.

Free Haemogregarine. The parasites usually are slightly curved, sausage or kidney shaped structures with rounded ends. (Plate V, figs. 1 to 8.)

The nucleus, which is situated in the middle or somewhat nearer one end of the parasite, is generally round or oblong in shape. Sometimes it consists of bands of chromatin extending transversely across the body, but more frequently it is made up of a compact mass of chromatin somewhat circular in shape.

The protoplasm, which is almost invariably stained a faint reddish or pink colour, never blue, is sometimes almost homogeneous but often it contains a mass of granules situated especially towards one or other, rarely both, poles. In many of the haemogregarines which are slightly narrower and more deeply stained, one end of the body is secured and this pointed end lies closely applied to the body. (Plate V, fig. 7.)

These more closely resemble the haemogregarines so commonly found in the blood of fishes.

Occasionally, especially in smears made from the lung, the haemogregarine is seen lying in a distinct capsule, and the margin of the latter extends in the form of a bow along the concave side of the parasite and is usually much more evident in the case of the parasites with a recurved pointed extremity. (Plate V, figs. 7 and 8.)

The whole parasite has then somewhat the appearance of a malarial crescent with its bow—the remains of a corpuscle. At first I was inclined to regard these forms as parasites situated in a red cell, but I think it more probable that the parasite is enclosed in a cyto-cyst as Miss Porter (1908) has described in the case of *Leucocytozoon musculi*. Another but very rare form of haemogregarine was occasionally found in smears made from the lung and liver.

This takes the form of a long, somewhat narrow, slightly curved shaped body, in which both ends taper and are rounded, but one end is sometimes more pointed than the other. The nucleus is oblong in shape and is situated slightly nearer the thicker end. (Plate V, figs. 4, 5, 6.)

The size of the free haemogregarines varies somewhat. The oval or kidney shaped forms usually measure about 10 μ long by 4·5 μ broad, and their nucleus about 6 μ in length and 4·5 μ in transverse diameter.

The long slightly curved parasite measures from 16 to 18 μ long and only about 3 μ in width, whilst the nucleus averages about 8 μ in length and 3 μ in transverse diameter.

Intraleucocytic Haemogregarines. Parasites contained within the protoplasm of mononuclear leucocytes were found in heart blood, liver and lungs—but were most numerous in the heart and lung. (Plate V, figs. 9, 10, 11.) The enclosed haemogregarine is nearly always of the sausage or kidney shaped form, and it evidently produces a marked karyolysing action on its host. In most cases the parasite has invaded and split up the nucleus, and the protoplasm has become discolourised and in some cases almost invisible.

Very often the parasite lies between portions of the nucleus of the leucocyte, and it is usually surrounded by a narrow margin of uncoloured area, probably a cyst.

Multiplication cysts of Haemogregarines were found in very small numbers in the heart blood, liver and lung. These are oval, rarely round, structures surrounded by a narrow unstained halo—the capsule. (Plate V, fig. 12.)

The average measurement of ten cysts was 25 μ by 18·7 μ.

The cysts are invariably stained a distinct blue colour with Giemsa and contain either partially differentiated protoplasm, or a number of definite blue stained merozoites, or not infrequently merely granular debris. It was difficult to determine the actual number of the merozoites as they were usually somewhat ill-defined, but I should say they varied from 8 to 20.

From the limited amount of literature at my disposal I think that both the *Toxoplasma* and *Haemogregarine* found together in the organs of this squirrel are new species, and although a few haemogregarines have been found in mammals in England—amongst these that of the field vole and field mice which I have previously described—yet apparently no *Toxoplasma* has been previously recorded in animals in this country.

It is reasonable to suppose that in this particular squirrel the presence of the *Toxoplasma* in such large numbers might have been the direct cause of death.

I have proposed that these parasites should be termed *Toxoplasma sciuri* and *Haemogregarina sciuri* respectively.

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DESCRIPTION OF PLATES II–V.

PHOTOMICROGRAPHS. *Magnification* = precisely 1000 diameters unless otherwise stated.

PLATE II.

Fig. 1. Fusiform trypanosome of Blackbird.

Fig. 2. Piriform trypanosome of Blackbird.

Fig. 3. Small trypanosome of Thrush.

Fig. 4. Large trypanosome of Thrush.

Fig. 5. Trypanosome of the Jay.

Fig. 6. Trypanosome of the Mole. Note variations in size of the three which are of precisely the same magnification.

Fig. 7. Two trypanosomes of the Rat.

Fig. 8. Two trypanosomes of the Pipistrelle.

Fig. 9. Immature stages of the trypanosome of the Pipistrelle. ×1500. One is pear-shaped and has two flagella proceeding from the centrosome. The other has two nuclei, two centrosomes and a pair of flagella to each centrosome.

Fig. 10. Immature stages of trypanosomes of the Pipistrelle, each has a single flagellum.

Fig. 11. Immature stages of trypanosomes of the Pipistrelle, one with two flagella, and a dividing one with four flagella.

Figs. 12 & 13. Pneumocysts from lung of Rat. No. 12 × 2000. No. 13 × 1500.

Fig. 14. Three haemogregarines from the Field Mouse. The third one is shedding granules, the middle one shows, unfortunately indistinctly, longitudinal divisions of the nucleus by myonemes.

Fig. 15. Multiplication cyst, somewhat degenerated, of the haemogregarine of the Field Vole.

Fig. 16. Haemogregarines (free) from the Rat.

Fig. 17. Haemogregarines (5) from the Field Vole. The top left is closely applied to the nucleus, the middle one is lying between the lobes of the nucleus and one free form is seen.

Fig. 18. Pigmented intracorpuscular parasites. Haemamoeba, of the Pipistrelle, showing various stages in growth. ×1500.

Fig. 19. Piroplasma-like intracorpuscular parasites (named provisionally *Nuttallia microti* sp. n.?) from the Water Vole. ×1500. The three in the middle row show division in cross form into four very small pear-shaped parasites. Note that all the parasites are somewhat marginal in position, one is almost outside the red cell. See also fig. 45.

Fig. 20. Piroplasma-like intracorpuscular parasites (named provisionally *Nuttallia muris* sp. n.?) from the Field Mouse. ×1500. Top right shows amoeboid changes; middle left and lower right a line passing across the centre, and the middle right a comma or pear-shaped parasite.

Fig. 21. Spirochaetes from the Pipistrelle (named provisionally *Spirochaeta vesperuginis* sp. n.). Compare 47.

Fig. 22. Spirochaetes from the Noctule, both 21 and 22 same magnification. Some of them show slight flattening and are thinner in the centre. Compare 48.

Fig. 23. Peculiar bodies, often found in the blood of the Mole.

PLATE III.

Figs. 24 to 26 and 28. Halteridia of Thrush: fig. 24, two microgametes; fig. 25 = two macrogametes; fig. 26 = young, and a mature macrogamete; fig. 28 = ookinete, with a microgametocyte (free flagellum) near.

Fig. 29. Halteridia of Starling, flagellating body—microgametocyte.

Figs. 27 and 30 to 44. Stages in leucocytozoon of Thrush.

Fig. 27. Two young leucocytozoa.

Fig. 30. Ookinete stage.

Fig. 31. Flagellating leucocytozoon of Thrush.

Fig. 32. Male and female (macro- and microgamete) leucocytozoa—note the rounded or spherical shape of the leucocytozoa of Thrush.

Figs. 33 to 44. Stages in the possible schizogony of the leucocytozoon of Thrush.

Figs. 37, 39, 40, 41 and 42. Show these multi-nucleated bodies inside the protoplasm of a leucocyte.

Figs. 33, 34, 35, 36, 38, 43 and 44. Free forms of the multiplication phase. The size and shape vary somewhat, as do the contained red chromatin masses. In many vacuoles are seen. Fig. 44 shows some of the nuclear masses escaping. Fig. 43 shows a dense collection of the red masses, whilst fig. 35 represents so dense an agglomeration of nuclear masses, which under the microscope appeared as densely stained red bodies, that it has come out in the photo as a homogeneous dark mass. One can see however the remains of the nucleus of the host cell.

Fig. 39. Possibly the youngest stage of schizogony.

PLATE IV.

Fig. 45. Segmenting cross-forms of piroplasm-like bodies, from the Water Vole (named provisionally *Nuttallia microti* sp. n.) × 1500.

Fig. 46. Dividing forms of the non-pigmented intracorpuscular parasite of the Pipistrelle, *Achromaticus*, to show its resemblance to the piroplasms. × 1500.

Fig. 47. Spirochaetes from Pipistrelle (named provisionally *Sp. vesperuginis* n. sp.). × 1500.

Fig. 48. Spirochaetes from the Noctule, × 1500: long forms some of which show indications of transverse division.

Fig. 49. Leucocytes from blood of Field Vole with cell inclusions.

Fig. 50. Graham-Smith's bodies, *Grahamella*, from very young Rat, showing intracorpuscular and free forms. × 1500.

PLATE V.

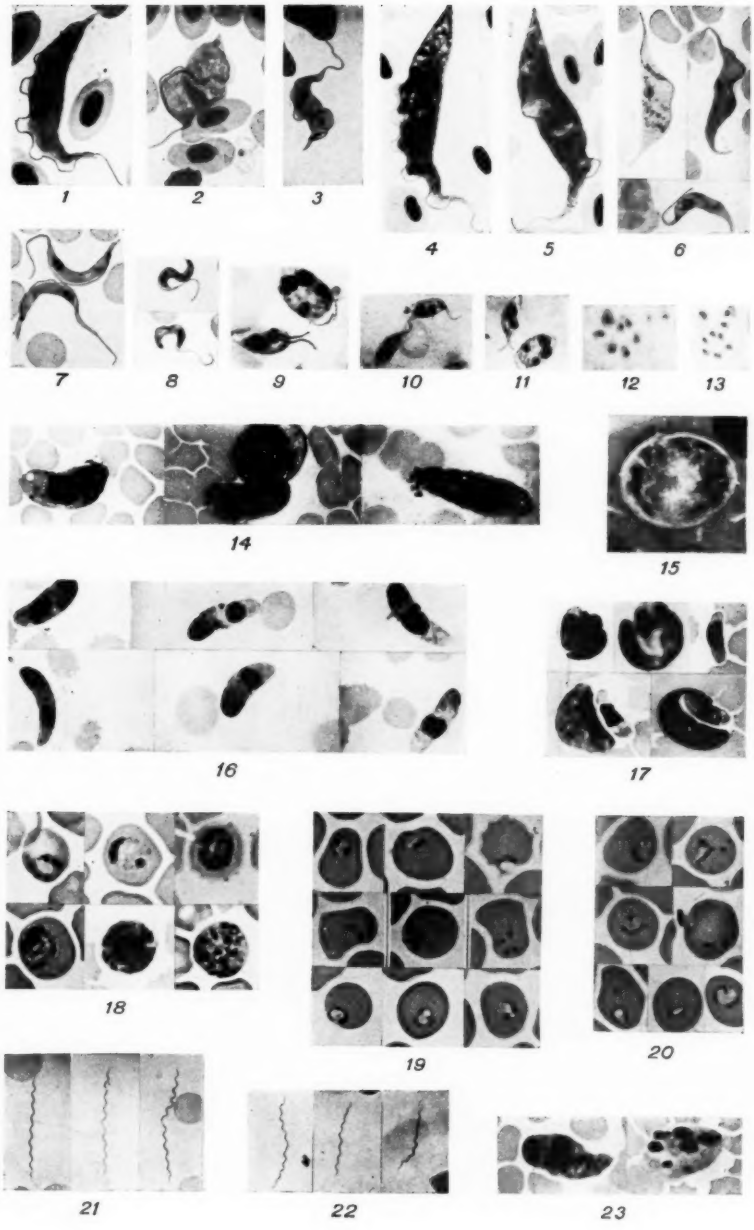
Figs. 1 to 12. *Haemogregarina sciuri* n. sp.

Figs. 1 to 8. Free Haemogregarines.

Figs. 1 and 2. Kidney or sausage-shaped forms fig. 1; showing especially polar granules.

Fig. 3. Somewhat longer form; figs. 4, 5, 6. Long, narrow and slightly curved forms.

Figs. 7 and 8. Haemogregarines with recurved pointed end deeply stained in a capsule.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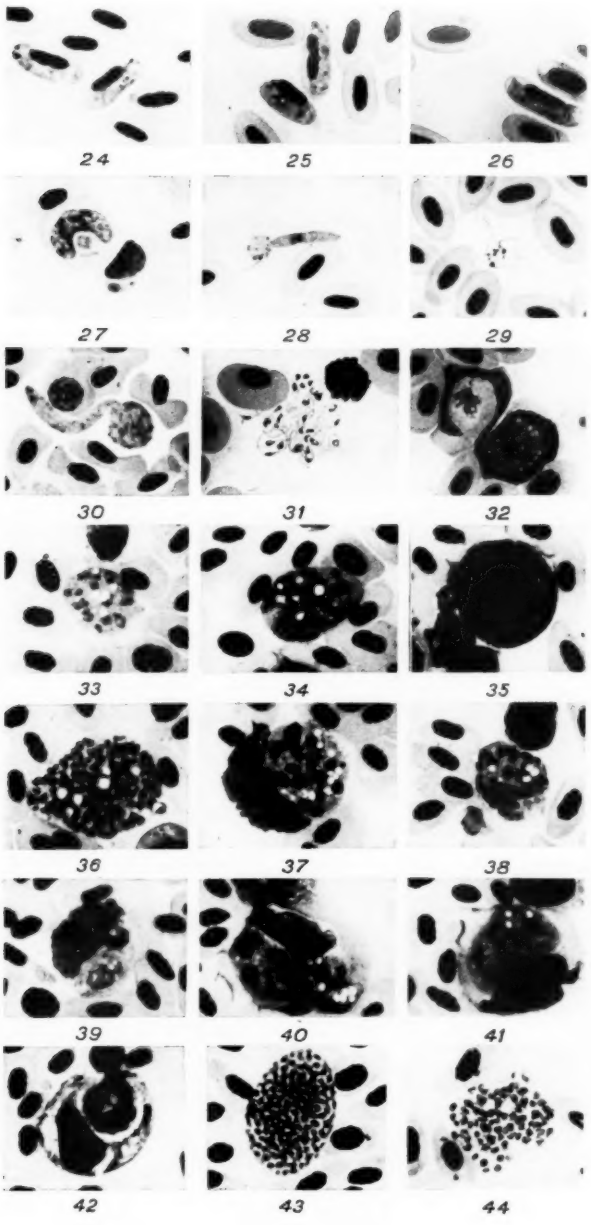
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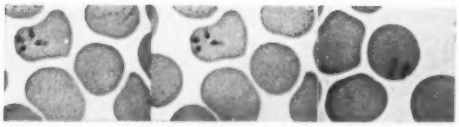

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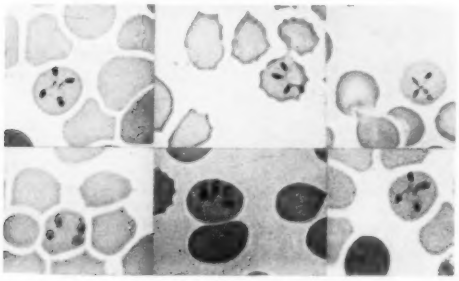

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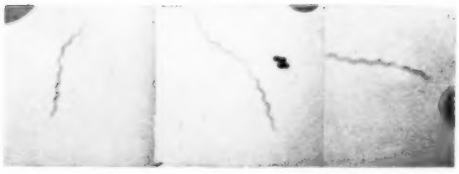

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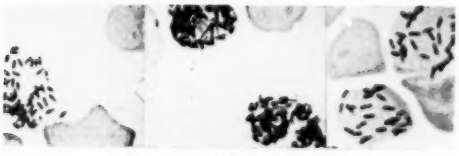

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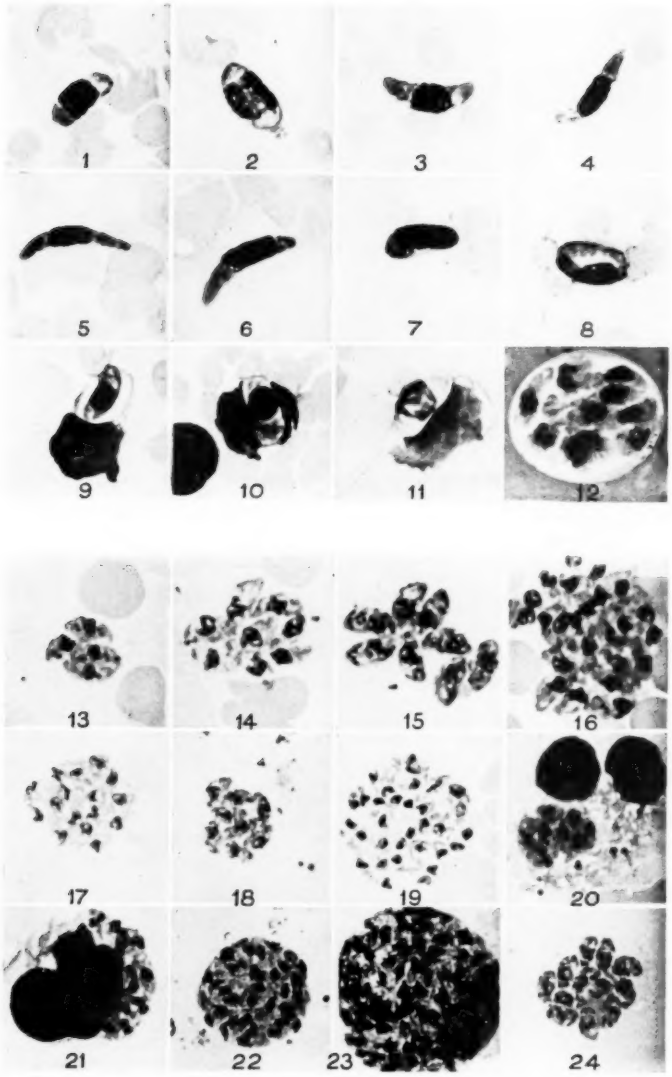
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Figs. 9, 10, 11. Haemogregarines enclosed within the protoplasm of a mononuclear leucocyte. In fig. 10 the parasite lies between lobes of the nucleus.

Fig. 12. Cyst of Haemogregarines containing about 10 merozoites.

Figs. 13 to 24. *Toxoplasma sciuri* n. sp.

Fig. 13. Group of four toxoplasmas, two of which are completely separated, the other two still attached. (With a lens it is possible to see a thin red line proceeding from the nucleus to a granule at the margin.) $\times 1500$.

Figs. 14, 15, 18 and 24. More or less loosely arranged groups of *Toxoplasma*. (Figs. 14 to 16×1500.)

Figs. 17, 19, 22, 23. Compact groups; figs. 22 and 23 somewhat resembling cysts, in which the outline of the individual parasites is more or less lost.

Fig. 20. Endothelial cell with two nuclei containing in its protoplasm a somewhat compact group of eight parasites.

Fig. 21. Mononuclear leucocyte containing separated toxoplasmas some of which are dividing.

THE TRYPANBLUE TREATMENT IN PIROPLASMOSIS OF DOMESTICATED ANIMALS IN SOUTH AFRICA.

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The following brief notes on the above subject are the outcome of the writer's experience in the use of Trypanblue in cases of piroplasmosis in animals. The inoculations were undertaken under strictly practical conditions in up country districts, and whilst no new discoveries are claimed, it was thought that this short description of the purely utilitarian employment of the drug might prove of interest.

Strength of Solution. The writer has used throughout a 2 % filtered and sterilized solution of the drug made with rain-water.

Dosage.

Dog: 5–30 c.c. (hypodermically) according to height and age.
Horses and Large Mules: 200 c.c.
Donkeys, Ponies and Small Mules: 150 c.c. } intravenously.
Cattle: 50–250 c.c. (according to age and size)

Diagnosis. Wherever possible blood smears were examined microscopically, but in many cases owing to the exigencies of field conditions diagnosis had to be made by clinical symptoms alone.

Biliary Fever of the Dog (Piroplasma canis.)

The writer has had excellent results with the drug in this disease, and would almost feel tempted to describe some of the recoveries as "miraculous."

The injection has always been made hypodermically on the chest wall, as great difficulty is often experienced in introducing a needle into the vein of the dog's thigh, especially when the animal is in the

anaemic state so often encountered in this disease. If an abscess forms at the site of injection—a not uncommon occurrence owing to the irritating nature of the drug, even when the most rigid aseptic precautions are observed—it never causes serious inconvenience if opened and treated in a suitable manner.

A Few Cases.

Case I. Subject. Crossbred Irish terrier, weight about 40 lbs.

When seen by the writer was in a very advanced state of the disease. Temperature 104·2°–105° F., pallid mucous membranes, and passing urine the colour of dark ale. Smears from the peripheral blood showed a heavy infection of the parasite and marked anaemic lesions. It was not possible to obtain the drug until the following day when the animal was in a state of collapse and literally unable to lift its head. 20 c.c. of the 2% solution were injected subcutaneously on the chest wall, more, it must be admitted, in the nature of an experiment than anything else. Twenty-four hours after the injection the animal was well "blued" all over, and the writer was astonished to see it barking and looking for food. An uninterrupted recovery was made without abscess formation.

Case II. Subject. Wire-haired terrier, weight about 25 lbs.

A typical clinical case well advanced, and smears showed a heavy infection. 15 c.c. of a 2% solution were injected on the chest wall. Twenty-four hours afterwards the animal was well "blued" and smears showed a great decrease in the number of the parasites. The subject made a good recovery and was an interesting case, inasmuch as the writer was able to take smears at twenty-four hour intervals for a week after injection to watch the disappearance of the parasites from the blood. None were seen after the fourth day and those which were encountered after the first twenty-four hours looked shrivelled and "sick." It was also noticed that the anaemic lesions in the blood quickly disappeared, until in a week's time it could have been described as "normal."

Case III. Subject. Bulldog, weight about 30 lbs.

Was noticed "off colour" for some days and gradually got worse; no marked clinical symptoms were observed for four days although the temperature gradually rose from 102° to 105° F., blood smears examined

each day were negative. On the fifth day the animal showed clinical symptoms, temperature 104·8°, pallid mucous membranes, urine highly coloured, and the dog refused all food. Although no parasites could be found after a very careful search the writer decided to inject 20 c.c. of Trypanblue solution. The animal made a slow but complete recovery after the formation of a large abscess at the site of injection.

This case is interesting, as, although no parasites were ever found in the peripheral blood, the animal responded to the drug. In this connection the writer has encountered a case in which parasites were never found in the peripheral blood, but were seen in great numbers in kidney smears after the animal's death.

Case. IV. Subject. Crossbred bull terrier, weight about 30 lbs., property of writer.

Developed typical "biliary fever" in Pondoland in November, 1911, showed marked clinical symptoms and a fairly heavy infection of parasites in the blood. Was injected with 20 c.c. of a 2% solution and made a good recovery after the formation of an abscess. The animal spent part of the next summer (tick season) in Pondoland and the remaining portion in a practically tick-free locality in the Karroo. In October 1913 the subject was taken to a very virulent biliary fever area near Cape Town, and in about twenty days developed all the clinical symptoms of the disease,—temperature 105° F., coloured urine, marked pallor of the mucous membranes, loss of appetite—although parasites were never found in the peripheral blood after a prolonged search in several films. 20 c.c. of a 2% solution were injected and the animal recovered satisfactorily. This case is interesting as showing that an animal can become infected a second time after an interval of two years, and that Trypanblue is efficacious in this second attack.

Equine Piroplasmosis, Biliary Fever (Nuttallia equi).

The writer was fortunate in being stationed for three years in a locality—East Griqualand—where "raw" horses and mules introduced from non-biliary fever areas generally developed the disease within six weeks of their arrival during the tick season. Biliary Fever in the horse is, of course, amenable to treatment by other drugs (*e.g.* calomel and chloride of ammonium), but the constant dosing three times a day for a week, which this treatment generally involves, is dangerous and inconvenient in all animals, and quite impracticable in the case of wild

or untrained ones. On this account the writer decided to give Trypan-blue a trial and he has been more than satisfied with the results obtained. So satisfactory has the treatment been in practice, and such is the confidence of the public in it, that many horses and mules are now brought into the district irrespective of the part they were bred in, which would have been considered a very risky proceeding a few years ago. This is a point of great practical importance to a community which has to depend almost exclusively on equine transport.

The writer has always injected the drug in large doses (200 c.c.) into the jugular vein of the horse and mule, and whilst taking precautions not to introduce air if possible, has been forced to the conclusion (through being compelled to use faulty syringes etc.) that the danger of injecting a little air into the vein has been greatly exaggerated. When ordinary aseptic precautions are observed the writer has never seen any ill effect follow the injection, beyond an occasional harmless oedematous swelling along the jugular furrow and on to the chest.

Two Typical Cases.

Case I. Subject. Carriage Horse, four years old.

This animal was brought to East Griqualand from a non-biliary fever area, and five weeks after its arrival it developed clinical symptoms of biliary fever (loss of appetite, mucous membranes jaundiced, urine stout-coloured, temperature 103°–104·5° F.). Blood smears showed marked anaemia and the presence of a few parasites, although it must be confessed that the author has nearly always experienced great difficulty in finding parasites in this disease in the horse and mule, in spite of the fact that all the clinical signs of the malady are present. 200 c.c. of a 2 % solution were injected into the jugular vein and the animal made an uninterrupted recovery, the temperature subsiding after twenty-four hours and the appetite slowly returning.

Case II. Subject. Imported North American Mule, aged six years.

This animal was brought almost direct off the ship into East Griqualand and was therefore very susceptible to the disease. Three weeks after arrival it developed biliary fever, but it had been noticeably ill for four days before it was seen by the writer. When examined it showed very marked clinical symptoms—*pallid* mucous membranes, temperature 104·8° F., hurried respiration, staggering gait, and generally

appeared to be in an almost collapsed condition—there was no opportunity to examine blood slides. 200 c.c. of a 2% solution of Trypanblue were injected into the jugular vein and next day a marked improvement had set in. The animal was eating a little and the temperature had subsided to 102·4° F., the mucous membranes were slaty-blue (they never take on such a bright blue colour in equines as they do in the dog after an injection of the drug); recovery was slow but complete.

The above typical cases in the horse and mule, out of some scores treated by the writer, will serve to demonstrate the quick and safe action of the drug. In his hands it has proved a most practical and efficacious treatment for this disease. But he trusts that it will not be brought into disrepute by being employed by unskilled persons as a panacea against every malady to which equine flesh is heir.

The writer has not encountered relapses after recovery although this is, of course, slower in some animals than in others, depending, in the writer's opinion, on the duration of the infection before treatment, the extent of the anaemic lesions present in the blood, and the particular susceptibility of certain animals. He has also never seen pneumonia supervening after the animal has been injected with Trypanblue, but this was a fairly common sequel with the calomel-chloride of ammonium treatment when the animal had apparently quite recovered from the piroplasmosis.

Piroplasmosis of Donkeys (Piroplasma caballi ?).

On account of the restrictions placed on ox transport owing to East Coast Fever in 1910, a large number of donkeys were introduced into the Mount Currie district of East Griqualand from all over South Africa—chiefly from non-biliary fever areas. As has already been stated, East Griqualand is a notoriously bad district for biliary fever in horses and mules, but these highly susceptible donkeys failed to contract piroplasmosis until they commenced going into Pondoland some eighteen months afterwards, when they soon developed a disease which the writer took to be "biliary fever." The explanation of the animals failing to contract the disease in East Griqualand, however, remained unsolved until his attention was drawn to a paper by Nuttall and Strickland (1912) in which the authors made a distinction between *Nuttallia equi* (Laveran) and *Piroplasma caballi* Nuttall, and he is now strongly of the opinion that these donkeys were susceptible

to *P. caballi* but not to *N. equi*. It must be confessed that the writer has never made any distinction in the shape or structure of the parasites, chiefly owing to the difficulties encountered in obtaining satisfactory smears, but he considers that the above statements are strong evidence in support of his view.

As the writer has never seen a description of the clinical symptoms of piroplasmosis caused by *P. caballi* perhaps the following brief account of a typical case of whatever disease these donkeys were suffering from, may prove of interest.

The first noticed sign is that the affected animal is seen standing in a dejected attitude with drooping ears and does not graze with the rest of the troop. When examined the mucous membranes are seen to be very *pallid* (not jaundiced as in the horse), the temperature ranges from 103°–105° F. or over, the urine is very discoloured (stout-coloured) and the animal has a general appearance of lassitude, depression and weakness. From the above description it will be seen that "biliary fever" is a distinct misnomer for this disease in the donkey, but it must also be borne in mind that the so-called biliary fever of the mule, when caused by *N. equi*, also gives rise to pallid mucous membranes and not the bile injected condition seen in the horse.

The writer from the first has employed Trypanblue for this disease in donkeys with excellent results, the dose used being 150 c.c. of a 2% solution intravenously for an adult animal, and feels that the lives of many of these useful little beasts have probably been saved which would have almost certainly been lost without the treatment.

South African Redwater in Cattle (Piroplasma bigeminum).

The writer has had few opportunities of testing the efficacy of the drug in the field in this disease, chiefly because cattle affected with the malady under ordinary South African conditions are generally almost *in extremis* before they are noticed to be ill. He has, however, had excellent results in a few cases, where it has been used to control a violent reaction with an artificially induced infection in imported cattle (protective inoculation).

Typical Case. Subject. Imported Friesland Bull, eighteen months old.

Injected with redwater and anaplasmosis blood obtained from Veterinary Research Laboratory, Pretoria. On the fourteenth day after

injection the temperature showed a marked rise in temperature. On the fifteenth day the animal was not feeding, respirations hurried, temperature 105·5° F., mucous membranes pallid, parasites present in small numbers in the blood. On the sixteenth day animal still not feeding, looking very depressed, eyes sunken, urine wine-coloured, temperature 106° F., parasites more numerous in the blood. 200 c.c. of a 2% solution of Trypanblue injected intravenously. Next day the temperature was down to 104° F., the animal began eating a little, looked brighter, and the parasites had greatly decreased in number and size. On the following day the animal had apparently quite recovered, temperature 102·8° F., eating well, mucous membranes bluish, no parasites found in the blood. The animal made an uninterrupted recovery.

General Conclusions on Practical Use of Trypanblue in Piroplasmosis.

(*a*) Parasites are not always present in the peripheral blood of dogs, and are often extremely difficult to find in other animals, especially under conditions encountered in the field. If well marked clinical symptoms are shown one is justified in using Trypanblue, even if parasites cannot be satisfactorily demonstrated.

(*b*) A large dose of a weak solution of Trypanblue should be administered. The writer cannot help thinking that the failures attributed to the drug by other observers are due, in many cases, to too small a quantity of the solution being injected.

(*c*) After an experience of the treatment in some hundreds of cases, the writer feels justified in stating that animals do not suffer from relapses if a sufficient quantity of the solution is used.

(*d*) The dog can become infected a second time after Trypanblue treatment, but this second infection is also amenable to the drug.

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THE SCOPE AND AIMS OF APPLIED ENTOMOLOGY[1].

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I. *Introductory Remarks.*

The class Insecta has existed from early Palaeozoic times, developing through the course of the ages into a numerical aggregate of species unknown and unparalleled in any other group of the animal kingdom. At the present day, there is every reason to believe that insect life is more abundant and luxurious than during any pre-existing epoch. With their remarkable adaptations to almost every conceivable environment, their still lengthier persistence on our planet is a matter of the greatest probability. Dr Holland has predicted in a word-picture how the class is destined to be the last to survive when all other forms of

[1] Being the substance of an inaugural lecture delivered before the University of Manchester on November 26th, 1913.

animal life have died out. He writes: "When the moon shall have faded from the sky and the sun shall shine at noonday a dull cherry red, and the seas shall be frozen over and the ice-cap shall have crept downward to the equator from either pole, and no keel shall cut the waters, nor wheels turn in the mills, when all cities shall have long been dead and crumbled into dust, and all life shall be on the last verge of extinction on this globe, then, on a bit of lichen, growing on the bald rocks beside the eternal snows of Panama, shall be seated a tiny insect, preening its antennae in the glow of the worn-out sun, representing the sole survival of animal life on this, our earth,—a melancholy 'bug'."

It can scarcely be regarded as remarkable that such a dominant class of animals should come into direct collision with the operations of man. Man has been forced to take into his cognisance the existence of such a warfare, and to take measures to ensure against his own defeat. Thus has the need for the recognition of applied or economic entomology taken birth. It is to be defined as the study of insects in relation to the welfare of the human race, and is divisible into three branches.

1. Medical entomology which concerns itself with those insects which attack man directly and thereby frequently transmit diseases which endanger his life.

2. Agricultural entomology which is the study of the insects which partially or completely destroy crops and forests or harm domestic animals.

3. Economic entomology, in the narrowest sense of the term, which deals with those insects which attack and destroy stored and manufactured goods.

II. *Medical Entomology.*

It is in connection with disease that insects inflict the most obvious direct injury to the human species. When it is reflected that the pathogenic agents of malaria, yellow fever, plague, sleeping sickness and many other virulent diseases are conveyed to man through the agency of biting insects, the importance of this aspect of applied entomology can scarcely be overestimated. The application of entomology to medicine has been crowned with some of the greatest triumphs of human ingenuity. It is now a well-known fact that by waging war against Anopheline mosquitoes the town of Ismailia has been practically freed from malaria. Prior to the discovery of the transmission of

yellow fever by means of the *Stegomyia* mosquito, the deaths from that disease in Havana rose as high as 1355 in one year. Two years after systematic mosquito destruction was inaugurated not a single case of yellow fever occurred in that town. We have, again, the striking

List of the principal diseases of man which, so far as known, are or may be carried by insects.

Disease	Distribution of the disease	Pathogenic organism	Carrier (or probable carrier)	Order to which carrier belongs
Malaria	Tropics and sub-tropics of the world	*Plasmodium* spp.	Anopheline mosquitoes	Diptera
Typhus Fever	Mostly in tropical and sub-tropical countries	... ...	*Pediculus vestimenti*	Rhynchota
Sleeping Sickness	Tropical Africa	*Trypanosoma gambiense*	*Glossina palpalis*	Diptera
Yellow Fever	Littoral tropics of America & Africa	... ...	*Stegomyia calopus*	Diptera
Bubonic Plague	Almost world-wide	*Bacillus pestis*	*Laemopsylla cheopus*	Aphaniptera
Kala Azar	Tropics and sub-tropics	*Leishmania donovani*	*Cimex rotundatus*? *C. lectularius*?	Rhynchota
Barbiero Fever	South America	*Schizotrypanum cruzi*	*Conorhinus megistus*	Rhynchota
Papatasi Fever	Tropics	... ...	*Phlebotomus papatasi*	Diptera
Dengue	Tropics and sub-tropics of the world	... ...	*Culex fatigans*? *Stegomyia calopsus*? *S. scutellaris*?	Diptera
Filariasis	Tropics of the world	*Filaria* spp.	*Culicidae*	Diptera
Elephantiasis	Tropics of the world	*Filaria bancrofti*	*Culex fatigans*	Diptera
Pellagra	Widely distributed in warm countries	... ...	*Simulium* sp.?	Diptera
Relapsing Fever	Cosmopolitan	*Spirillum obermeieri*	*Pediculus vestimenti*?	Rhynchota
Infantile Paralysis	Almost cosmopolitan	... ...	*Stomoxys calcitrans* *Tabanus lineola* or other biting flies?	Diptera
Verruga	S. America	... ...	*Phlebotomus verrucarum* Town.?	Diptera

instance of the newly opened Panama Canal. The French failed in that project owing to the enormous toll of human lives entailed through yellow fever, and to financial disaster. The toilers, from the engineers and doctors to the coolies, were afflicted with the dreaded disease. It is stated that out of 36 nurses brought over at the same time only 12 survived. During one month nine members of the medical staff of a single hospital succumbed. Out of 18 French engineers who were brought over together, with the exception of one all died from yellow fever within one month of their arrival (Doane, 1910, p. 136). When the U. S. Government took up the work they realised that neither money nor engineering skill was of any avail unless immunity from yellow fever could be attained. All areas of standing water in the immediate neighbourhood of the Canal zone were filled up. Labourers were enforced to live in mosquito proof buildings in order to escape being bitten by mosquitoes. To-day the workmen can even sit outside their houses in the evenings without being sheltered behind gauze screens, so effectually have mosquitoes been exterminated (Gossard, 1909, p. 320). It may be truly said that mosquitoes were the greatest problem in the cutting of the Panama Canal. In the year 1900 in India 4,919,000 people died from malaria and unclassified fevers. There is every reason to believe that the majority succumbed from the direct or indirect effects of mosquito bites. Recently mosquito surveys have been initiated in India and the scope and utility of the Sanitation Department extended. It is to be hoped that the Government of India at last realises the paramount importance of such work, and the futility of attempting to govern peoples successfully so long as the bulk of their members suffer from preventable disease, and lack of adequate sanitary measures.

III. *Agricultural Entomology.*

It is, however, in connection with agricultural entomology that I propose to devote the greater part of this article. Insects affecting agriculture present to us an extraordinary diversity of problems. If phytophagous insects were able to multiply in accordance with their natural powers of reproduction, they would be able in a few years to destroy the greater part of terrestrial vegetation. Fortunately there are a number of controlling agencies which effectually restrict the multiplication of such insects. Among these various factors are climatic conditions, insectivorous birds, scarcity of food supply, fungi and other pathogenic organisms. The most important, however, are

other insects which are carnivorous instead of herbivorous in their feeding habits. Under artificial conditions induced by cultivation the balance of Nature becomes disturbed. Agricultural operations largely consist in planting certain plants to the exclusion of others, over continuous areas of country. The insects which live at the expense of these plants are thus provided with conditions eminently favourable to their excessive multiplication, and become pests to the agriculturalist. The result is that the efficacy of the normal controlling factors of these insects becomes greatly reduced. Man has to step in and devise methods to take their place. It is the aim and function of applied entomology to investigate the habits and life-histories of all noxious insects. The ultimate object of such work is to provide the requisite knowledge upon which to base those remedial or preventive measures which are likely to be effective in each specific case.

The agricultural pests of this country do not cause less financial loss in proportion to the area under cultivation than in any other country. We have the advantage in England that no extensive areas of country are devoted to the cultivation of single crops to the exclusion of others. It is these conditions that offer unlimited facilities for the multiplication of the insects subsisting thereon, and have led to the vast problems that have to be faced in America, India, and other lands. If we were in possession of an adequate knowledge of the chief insects affecting English agriculture, the work of successfully combating them would make more rapid progress. I can see no valid reason why the damage they commit should not ultimately be reduced to an almost negligible quantity. It is essential to make an exact study of the life-history and biology of each individual species of injurious insects from every point of view. It is further necessary to study its seasonal occurrence, its distribution abroad, its parasites and the insects and other animals that are co-existent with it within the same area. The frequent failure of the measures recommended to combat insects in this country is more often than not traceable to lack of knowledge of the economy of the particular species being dealt with. Kieffer has called attention to a remarkable fact in a Cecidomyiid, *Diplosis tritici*, which attacks the grains of wheat. One of the measures advised, namely, burning the debris after threshing, has only an injurious effect. While it is true that this debris contains pupae of the *Diplosis* it is noteworthy that the healthy and non-parasitised larvae of these flies transform beneath the soil, while those which remain in the heads are, on the contrary, parasitised (Marchal, 1907, pp. 14–15). Miss Embleton

(1902, p. 221) has pointed out that a colony of scale insects may be extensively parasitised and yet betray no obvious manifestation of the fact except to the trained and experienced eye. In such case it is, as a rule, highly injurious and superfluous to apply insecticides as they also destroy the beneficial insects which are already keeping down the abundance of the injurious species. These two instances may serve to illustrate the importance of a scientific knowledge of the insects we deal with. A too prevalent belief is that the methods of applied entomology only concern the destruction of insects. An equally important side is the preservation and encouragement of the vast numbers of species which are directly beneficial to the agriculturalist. Measures for dealing with an insect pest may therefore be divided into

1. The utilization of natural methods of control.
2. The adoption of artificial methods.

(*a*) *Natural Methods of Control of Injurious Insects.*

These for the most part consist in the preservation and increase of those organisms which are definitely beneficial in that they destroy injurious species of insects. They mainly comprise carnivorous insects and insectivorous birds[1]. Carnivorous insects may be divided into predaceous species and parasitic species. (1) *Predaceous insects* are those which attack other insects directly and devour them. Immediate benefit is thus derived from their action. They comprise a very large number of species including Coleoptera belonging to the families of the Çicindelidae, Carabidae, Staphylinidae, Coccinellidae together with the Cleridae, Cucujidae and several minor groups. The larvae of the Dipterous families of the Syrphidae and Leptidae, and the Neuropterous family of the Hemerobiidae are likewise actively predaceous. Similarly many Rhynchota, also Mantidae, Empidae, Asilidae and Vespidae are likewise beneficial. All such insects, some more so than others, directly aid the agriculturalist. (2) *Parasitic insects* which deposit their eggs in the bodies of other insects or in their immediate neighbourhood. They pass the greater part of their life within their hosts whose death they sooner or later bring about through devouring their internal tissues. Parasites amount to tens of thousands of species and constitute Nature's most effective method of control over the excessive multiplication of insect life. They are almost exclusively confined to the orders of the Hymenoptera and Diptera. Among the

[1] For the rôle birds play in connection with agricultural entomology, *vide* Newstead (1908). Also Collinge (1913) and F. V. Theobald (1907).

former, the families of the Chrysididae, Braconidae, Chalcididae and Ichneumonidae, with their allies, comprise an enormous assemblage of species. In the Diptera, the chief parasitic families are the Bombyliidae, Tachinidae, Pipunculidae, and Conopidae. The Oestridae and Hippoboscidae, on the other hand, are directly injurious, as they include parasites of domestic animals.

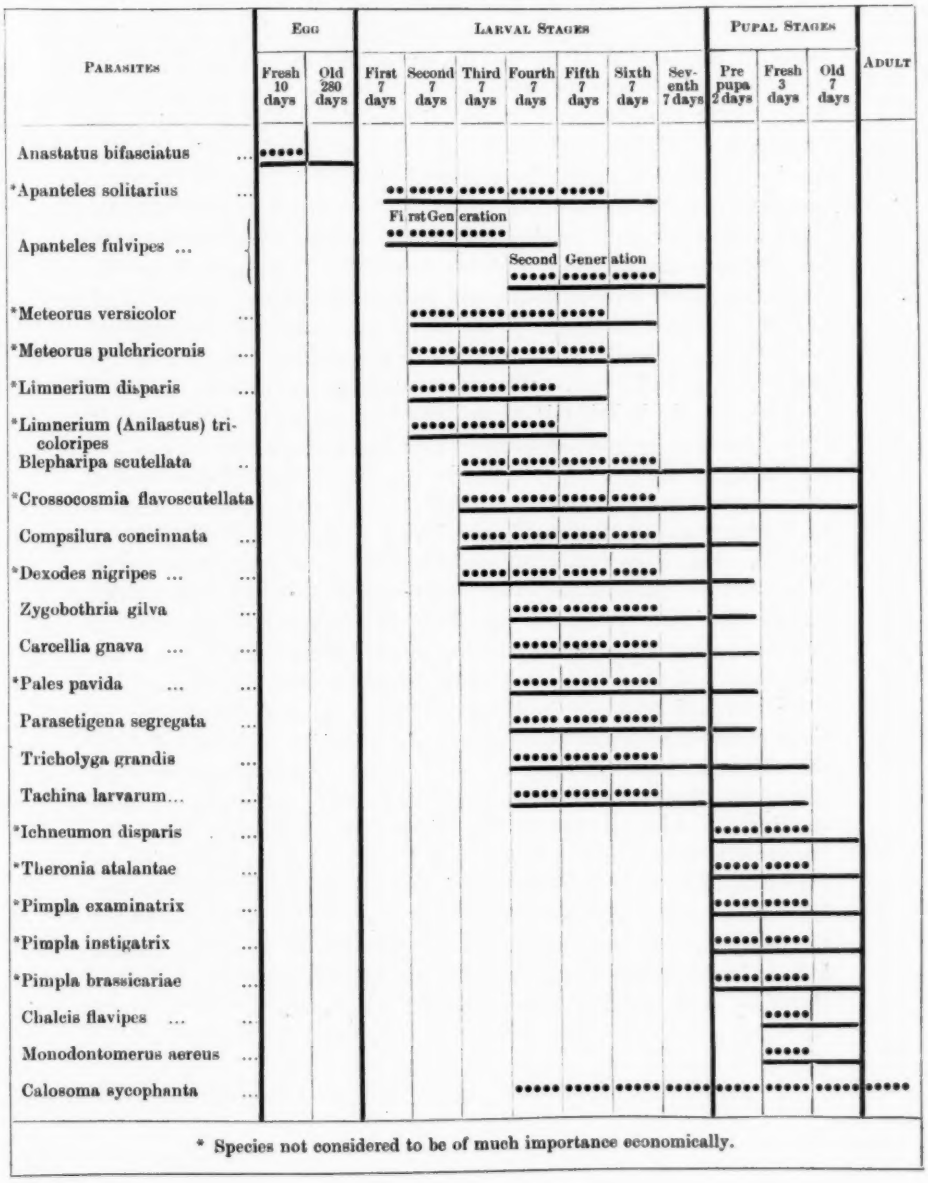
Frequent instances are known where an insect has been highly injurious for several years, and increased to the verge of becoming a veritable plague. It has afterwards disappeared, as if by magic, and may not again attract notice as a pest for many years. Experience has shown it is almost always to the work of parasites that the rapid disappearance of injurious species must be attributed. Prof. Paul Marchal (1907, p. 8) states that the damage caused by the larvae of moths of the genus *Hyponomeuta* to fruit trees, is almost invariably stopped at the end of 2 or 3 years by Tachinids and other parasites. The same thing occurs with the larvae of Bombycid moths which defoliate coniferous forests. The outbreaks of the Hessian fly and oat midge in France in 1895 were brought to a termination through the destruction wrought by their parasites. Such outbreaks, however, are not brought by Nature to a termination until the species concerned have passed through a number of generations, and committed widespread damage. Bellevoye and Laurent (1897, p. 71) have demonstrated numerically that it is unnecessary for a parasite to have a fecundity greater than that of its host. It is possible, in the course of three generations, for a parasite of equal fecundity to its host to destroy the latter completely, even though the parasites were outnumbered in the proportion of 3 : 1 at the commencement. Many experiments have been conducted with reference to the natural control of injurious insects by means of other species which prey upon them. In certain instances remarkable success has been achieved—in other cases failure or only indifferent success has resulted. The importance of this line of work can scarcely be overestimated. It cannot, however, be relied upon as a substitute to replace all the other methods commonly used in applied entomology, as has been advocated in some quarters. A large number of experiments have been made in the United States and on a smaller scale in Italy, Portugal, Canada, France, Peru, and other countries, but in England this line of work is practically a virgin field. The principle involved is that the injurious insects of a particular country may be present also in other countries, or parts of the same country, and yet not produce any serious damage therein. In such instances it has been

discovered that, in those countries where a pest is innocuous, its numbers are to a large extent kept down by parasitic insects which destroy it. By importing these parasites into a country, where the hosts are little affected by parasites, and therefore causing widespread damage, remarkable success in certain instances has been achieved. One of the most striking cases of this nature is the importation of the Coccinellid beetle *Novius cardinalis* from Australia into California, to control the orange scale *Icerya purchasi*, which was devasting the orange and lemon groves of that country (Howard and Fiske, 1911, pp. 24–27). All attempts to combat it by means of insecticides proved useless, but the imported beetle adapted itself to its new surroundings so effectually that, in less than one year, the scale insect was reported by Prof. Riley to be no longer committing serious damage. This was in 1889, and the scale is still kept effectually under control. It is further noteworthy that it has been similarly dealt with in Florida, New Zealand, Portugal, Cape Colony, Formosa, Egypt, the Sandwich Islands, Italy, Syria, and quite recently in France and Malta. In Italy, mulberry cultivation has been threatened by the scale insect *Diaspis pentagona*. In 1891 the pest assumed such serious proportions that the Italian Government passed a legislative measure compelling mulberry cultivators to use all available means of coping with it. This, however, proved of little efficacy, and it was not until Prof. Berlese of Florence introduced a species of Chalcid fly (*Prospatella berlesei*) from America into Italy that much headway was made. The results of the importation have proved most successful and Prof. Berlese (1913, p. 697) believes that by the end of the year 1914 the *Diaspis* scale will no longer be a serious enemy to Italian fruitgrowers.

In the Sandwich Islands the sugar cane has suffered serious depredation from leaf-hoppers accidentally imported there from Australia. In 1904 Perkins and Koebele visited Australia in order to investigate all likely parasites of the leaf-hoppers. Over 100 species of these parasites were brought to light as the result of their work, and five species thereof are stated to be acclimatised in Hawaii. They are all minute Hymenoptera, preying upon the eggs of the leaf-hoppers, and the practical results of their importation are stated to be quite satisfactory (Howard and Fiske, 1911, p. 35). Another interesting example of successful control is found in the case of the cotton worm (*Alabama argillacea*) in the island of St Vincent. The insects which appear chiefly responsible for the destruction of the cotton worm are the wasp *Polistes annularis* L., a Chalcid fly—probably *Chalcis annulatus* Fabr.—

Sequence of Gipsy-moth Parasites in Europe.

(From Howard and Fiske, p. 132.)



* Species not considered to be of much importance economically.

and the predaceous ground beetle *Calosoma caldium* Fabr. Of these, the *Polistes*, which is predaceous on the larvae of the cotton worm, is stated to be the most important (Ballou, 1907, p. 235; 1912, p. 315). The most extensive campaign yet attempted against any destructive insect is that organised by the Bureau of Entomology of the U. S. Dept. of Agriculture with reference to the Gipsy moth (Howard and Fiske, 1911; Fiske, 1913). Introduced from Europe into Massachusetts in 1868 it multiplied and spread with excessive rapidity, owing largely to the fact that although in Europe it is held in check by various parasites, these latter were not introduced into America along with the host. The work of Fernald and Forbush, in 1896, demonstrated that the Gipsy moth is attacked in America only to a very small degree by indigenous species of parasites and predaceous insects. They found that the maximum numbers of the Gipsy moth destroyed by parasites was 10%, and that on an average it was nearer 2%. Studies on the same lines, made in several European countries, revealed the fact that over 80% of the individuals are destroyed by parasites. In the case of the Gipsy moth, as well as in many other Lepidoptera whose larvae are leaf-feeding, that have been studied in America, it is found that control by means of parasites must come about through the agency of a variety of species working together harmoniously (Howard and Fiske, 1911, p. 105). This is an important generalization, as certain other species of destructive insects have been successfully controlled through the agency of single species of parasites. It is, therefore, the aim in America to secure the firm establishment of a sequence of the egg, the caterpillar, and the pupal parasites of the Gipsy moth under analogous conditions which obtain in Europe. The study and importation of these parasites have now been going on for a number of years and, until the object is either achieved or proved to be impossible, Dr Howard states that the fight will not be abandoned. On p. 77 is given a table showing the chief parasites of the Gipsy moth in Europe. Opposite the name of each parasite is a dotted line showing the stages in the life of the Gipsy moth during which it may be attacked by the parasite in question. The solid lines denote the stages in its life during which the Gipsy moth is likely to contain the parasites in its body. At the head of each vertical column is stated the approximate number of days the Gipsy moth spends in each stage of development.

(*b*) *Artificial Methods of Control.*

These are for the most part concerned with the application of chemical agents known as insecticides—agents which are used with the object of directly killing noxious insects. They may be divided into three classes:

1. Stomach poisons.
2. Contact poisons.
3. Deterrants.

1. Stomach poisons are applied to the leaves of plants with the intention of directly poisoning the insects feeding thereon. Of such substances Bordeaux mixture and various compounds of arsenic are in general use. The best known arsenical preparations are copper aceto-arsenite ("Paris Green"), lead arsenate, copper arsenite ("Scheele's Green"), arsenite of lime and arsenite of soda. These substances are highly destructive to insect life, and have the advantage of being efficient when used in extremely small proportions. According to Maxwell-Lefroy and Finlow (1913, p. 270) a plant sprayed with lead arsenate, for instance, using 1 part by weight to 1000 of water is poisonous to many insects; at a strength of 1 in 500 (1 lb. to 50 gallons of water) it renders plants poisonous to all leaf-eating insects. Copper aceto-arsenite is still more virulent, and can be used in even smaller proportions. Such compounds have the objection of being poisonous to cattle should a certain standard strength be exceeded. Theobald (1909, p. 515) states that fruit trees should not be sprayed with them when in flower as bees are readily poisoned, and they should be applied at least one month before the fruit is gathered for consumption. Among other stomach poisons, lime-sulphur, both alone and combined with reduced strengths of arsenate of lead, has proved successful in certain instances in America (Scott and Siegler, 1913). Maxwell-Lefroy and Finlow (*loc. cit.*) have conducted experiments with lead chromate in India, and they claim it to be a better stomach poison than lead arsenate for that country. It has the advantage of being devoid of arsenical compounds, does not affect the plants, is not easily washed off from the leaves, and its yellow colour renders sprayed plants very easy of recognition. It remains, however, to be ascertained how far it will prove useful in this country.

An ideal insecticide, acting as a stomach poison, should be insoluble to the action of rain, dew, or other atmospheric conditions, cheap and effective at weak strengths, and non-injurious to the plants.

2. Contact poisons comprise a very wide range of chemical substances which are used either as sprays, washes, fumigants, or soil dressings with the object of killing injurious insects by means of surface contact with the latter. They may be divided into :—

a. Soaps and various alkalies.

b. Pyrethrum and alkaloids of tobacco and other plants.

c. Various phenol compounds and mineral oils.

d. Rosin, and lime and sulphur compounds.

e. Poisonous gases such as hydrocyanic acid and bisulphide of carbon.

It may be added that there is a very wide field of research open for an experimental study of the physiological action of these substances. Does the egg, larva, pupa, or perfect insect furnish the best opportunity for their application? Further, if a compound is effective against one species of insect it by no means follows that it is equally effective for many others. A leaf-feeding larva may be killed by poisoning the plant it feeds upon, but sucking insects, which subsist upon the nutritive juices drawn from the interior of the plant, can usually only be dealt with by means of contact poisons. In some cases these insecticides kill by means of direct surface contact by "burning" the skin. In other instances they affect the respiratory apparatus by forming a coating over the body, thus closing up the apertures of the spiracles. Whether they ever enter the respiratory system in other than the gaseous state is a doubtful point. On account of the small size of the spiracles, the extremely elaborate closing and guarding apparatus with which the latter are provided, and the narrow calibre of the tracheal branches, it seems doubtful whether insecticides (in the form of liquids or powders) could ever enter the respiratory system in sufficient quantities to be effective. Maxwell-Lefroy (1906, p. 81) states that kerosene emulsion acts partly by mechanically closing the spiracles thereby inducing asphyxiation; possibly it also has a poisonous effect on the tissues of insects when its vapour is gradually absorbed through the tracheal system. Dewitz (1912, p. 352) is inclined to believe that contact insecticides, both in the liquid and powder form, have a fatal effect on the sense organs of insects. He quotes the statement of Fujitani (1903, p. 47) that the active agent of pyrethrum is a nerve and muscle poison to which fishes and insects are very sensitive. The physiological action of soil dressings is likewise not understood. The whole subject of the physiological effect of insecticides has never been adequately studied; a much more exact knowledge of the subject is necessary before many

of the latter can be confidently recommended to the agriculturalist In this country, the best work so far prosecuted on insecticides, is the series of experiments carried out at the Woburn Experimental Fruit Farm (Duke of Bedford and Pickering, 1906, 1908 and 1909).

3. Deterrants may be defined as poisons usually of a milder nature than contact or stomach poisons, and not necessarily insecticidal in their action. Crops are sprayed with these agents in order to render them nauseous, with the result that they are saved from insect depredations, the insects being compelled to seek other pabula.

IV. *Economic Entomology.*

The term "economic" entomology I propose to restrict to its narrowest sense namely, with reference to those insects destructive to stored and manufactured goods. Under this heading we have all insects injurious to grain, flour, nuts, seeds and other dry food-stuffs. Insects attacking woollen goods, carpets, furs, leathers and hides; also those species injurious to paper, books, stored timber and furniture; insects attacking cheese, hams, tobacco, dried fruits, vinegar, drugs and museum specimens. In short this branch of the subject might be defined as commercial and domestic entomology. The possibility of the utilization of the X-rays in connection with this branch of the subject has not so far been adequately tested. Forel and Dufour subjected ants to the Röntgen rays, but they exhibited no tendency towards being affected thereby, and no after-effects were detected eight days after the experiments had been discontinued. Axenfeldt experimented with houseflies, and concluded that they can perceive the X-rays, and that the latter affect them in much the same way as ordinary light. Hunter (1912, p. 188), in America, subjected insects in various stages of development to the action of the X-rays, but no effects were detected upon the fertility or the development of the species used. In his experiments special attention was directed toward the determination of the question whether the sexual organs of insects are affected in any manner analogous to that in the case of mammals, but the results gave an entirely negative answer. In April of last year, Morgan and Runner (1913, p. 226) published records of a careful series of experiments on the effect of the X-rays on the cigarette beetle, giving full details of the methods employed. The results, however, were also entirely negative; the subsequent behaviour of the insects was in every way identical with that of the untried individuals used as controls. In

view of the fact that the X-rays have a decidedly injurious effect upon certain tissues and functions in the higher animals, these results are somewhat remarkable. The possibilities, however, of this line of investigation cannot yet be said to be in any way adequately tested. Furthermore, certain of the associated rays require isolating and their effects definitely ascertained.

V. *The place of Physiological Research in Applied Entomology.*

In addition to the physiological questions already discussed, there is a very wide field for the investigation of the tropisms of insects, especially chemotropism or, in other words, their orientation to olfactory sensations. This phenomenon is particularly in evidence in the selection by larvae of their food-plants, by the female when she selects particular plants for the purposes of oviposition, and in the pursuit of the male for the female. Quite recently Tragardh (1913, p. 113) in Sweden, and Dewitz (1912, p. 343) in Germany, have emphasised the possible importance of the study of chemotropism in relation to applied entomology. Verschaffelt (1910, p. 536) has investigated the factors which determine the selection of food in the case of the larvae of *Pieris brassicae* and *P. rapae.* The larvae choose as their food-plants certain of the Cruciferae, also *Tropaeolium* and *Reseda.* In these plants there occurs a group of glucosides—the mustard oils. Verschaffelt took a solution of sinigrin, which constitutes the glucose agent in black mustard, and uniformly distributed it over the leaves of plants which the *Pieris* larvae had previously refused to eat. Leaves so treated were devoured readily. From such experiments it appears that the *Pieris* larvae exhibit strong chemotropism towards mustard oils, and it is due to their presence in the leaves of certain plants that determines the selection of the latter by the larvae for their food. By a similar method of research, Verschaffelt has shown that the larvae of the saw fly, *Priophorus* (*Cladius*) *padi*, which feed on certain of the Rosaceae, are attracted by the glucoside known as amygdaline. As regards the factors which determine the females of many insects to select certain plants for purposes of oviposition, definite information might be acquired by carrying out an analogous series of experiments. Howlett (1912, p. 416) has recently experimented with certain Diptera, and in the case of the stable fly, *Stomoxys calcitrans,* he succeeded in inducing the female to lay her eggs on pieces of cotton wool soaked with valerianic acid—a substance occurring in decaying vegetable refuse. The obvious

application of such researches lies in the possibility that, in some instances, they may be utilised in their preparation of lures to entice the females, in virtue of their chemotropic properties, to deposit their eggs thereon. Such eggs could then be readily destroyed. If devices of this nature were in existence, methods of combating the larvae after damage had already been committed would no longer be necessary. Howlett (1912, p. 412) has also conducted some experiments in India with the fruit fly, *Dacus zonatus*. He selected a number of essential oils and found that citronella oil was remarkably attractive to that species. He furthermore noted that only males were attracted. He mentions that in less than half an hour a handkerchief, moistened with this oil and exposed in a peach orchard, became almost hidden by the multitude of the insects attracted thereto. As the reaction was confined to the male sex, and did not in any way appear connected with feeding habits, he formulated the hypothesis that the odour of citronella oil resembled that emitted by the females to guide the males to them. Dewitz ascertained that it is the odour of the nectaries of the vine flowers that attracts the vine moths and induces them to oviposit on the unopened buds. Labergerie (1911, p. 612) conducted a series of experiments with molasses diluted with water, and exposed in a vineyard in 3000 earthen vessels and 200 metal ones. By this method, between the 18th of May and 15th of September, there resulted the capture of 290,000 vine moths and their larvae at small expense. Barrows (1907, p. 515) has made a study of the chemotropic reactions of the pomace fly, *Drosophila ampelophila*. This insect occurs in great numbers around cider-presses, packing sheds and orchards, when fermenting fruit is present; within the latter it deposits its eggs and its larvae develop. He studied the reactions of the fly to various substances occurring in fermenting fruit. Ethyl alcohol, acetic ether, and acetic and lactic acids were experimented with separately and in mixture. The insect was found to be attracted in the most marked degree by a mixture of ethyl alcohol of 20% strength and acetic acid of 5%. It was further discovered that cider vinegar and fermented cider contain alcohol and acetic acid in percentages very close to those just quoted. A series of experiments were conducted proving that the fly discovers its food by means of the olfactory sense and this sense is located in the terminal joint of the insect's antenna.

These instances have been enumerated with the object of pointing out what an abundant field exists for physiological research as regards chemotropism. It is a line of work that is likely to ultimately achieve

important practical results which might be applied, in many instances, at no great cost of labour or outlay of money.

The importance of another series of tropisms, namely phototropism, has also been recently emphasised by Dewitz (1912, p. 343). He mentions that the reaction of insects to various luminants was first made use of practically by the Abbé Roberjot in 1787. The Abbé endeavoured to destroy the vine moth (*Oenopthira pilleriana*) by means of lighted candles placed in the windows of his house, and by wood fires in the vineyards. Since that time, this method has been extensively developed in France, and in the vineyards of Champagne an elaborate installation of electric lights is used for trapping and destroying insects destructive to the vine. Much ingenuity has been expended in devising light traps, but the fundamental scientific aspect has been neglected. It is important to ascertain the optimum candle power desirable, and which portion of the spectrum exercises the most marked attraction to insects. It is further desirable to make a spectroscopic examination of the various kinds of lights likely to be used, and whether the reactions of various insects differ in a marked degree from one another. Perraud (1904, p. 619), so far, is the only investigator who has paid much attention to this side of the subject. Projecting a large spectrum on a screen in a dark room, he noted the segregation of certain species of Lepidoptera on the different colours. The red to the green portion of the spectrum exercised by far the greatest attraction. White lights, however, proved more attractive than any single isolated colour. Dewitz's experiments (Dewitz, 1912 A), by means of lamps provided with glass screens of different colours, gave other results, as he found that the green proved the most effective. Dewitz and Vermorel (*vide* Dewitz, 1904) have conducted a long series of experiments with acetylene lamps, in order to determine the proportions of the sexes among Lepidoptera that were attracted. In the family of the Bombycidae only 4 % of the moths attracted were females, among the Noctuidae 19 %, in the Geometridae 27 %, and in the Tineina 39 %. Dewitz and Gastine (*vide* Gastine, 1903, p. 630) found that in the case of the moth *Porthesia chrysorrhoea* there was a good deal of variation in the proportion of the sexes attracted. This was believed to be due to varying atmospheric conditions. Observations conducted by David and Laborde (Abstract in *Rev. Applied Entom.* Ser. A, Vol. 1, 1913, p. 9), using 1200 lanterns over an area of 270 acres, for a period of 17 days resulted in the destruction of about 100,000 various Lepidoptera of which approximately 40 % were females. Although it was computed that only

about 20% of the females taken had not deposited their ova, 496,000 possible larvae were thus prevented from coming into existence. The total cost of the experiment was stated to be £64.

VI. CONCLUSION.

I have endeavoured to show in the brief space of this article what the aims of applied entomology are. How wide the field is for the higher forms of scientific research. The conceptions of the scope of entomology, in relation to agricultural development, have gradually but surely expanded in all civilised countries. At the present day, a worker in this field finds himself involved in problems of a vastly wider range than the superficial anatomy of a common insect pest or the compounding of a standard insecticide. To carry out this line of work, in order that it may ultimately benefit the State, requires men properly trained in methods of research. Adequately fitted laboratories are necessary, with funds for their annual maintenance. Ready access to affected districts where problems can be studied in the field is also essential, and it is mainly from a combination of laboratory and field work that success is most likely to ensue. State recognition of the value of entomology has recently made considerable progress in this country. The formation of the Imperial Entomological Bureau marks the most important step yet taken in Europe towards official recognition of the subject. The establishment of a chair of entomology at the Imperial College of Science, the appointment of an entomologist to the Board of Agriculture, and the founding of a state-aided department of Agricultural Entomology at Manchester University augur favourably for the future of the subject in England.

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EQUINE BILIARY FEVER IN MADRAS.

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Introduction.

In no part of India, perhaps, are ampler opportunities available for the study of tropical diseases than in the Presidency of Madras. This will be borne out by the fact that quite recently the interesting discovery was made in the Madras Veterinary College Hospital of the existence in these parts of two distinct parasites, causing the protozoal biliary fever among horses. I propose in the course of this article to chronicle the details of my observations with regard to this discovery, feeling sure that they will be read by all Veterinarians with interest, though they may probably arouse a feeling of melancholy among lovers of horse flesh. There is no disguising the fact, that through lack of facilities for prosecuting the study of tropical diseases in Madras the Veterinary Profession has been greatly handicapped. It is therefore with no little satisfaction that we hail the announcement made by Sir Harold Stuart, member of the Executive Council of the Government, at a recent public function in Madras, that at no distant date there would be a fully equipped School of Tropical Medicine in the City.

Nuttall and Strickland (1912) have shown that two distinct parasites and consequently two distinct diseases occur in horses suffering from "biliary fever" and have named the particular infections, which I am now dealing with, after the parasites which produce them, *i.e.* Piroplasmosis due to *Piroplasma* or *Babesia caballi* Nuttall (1910) and Nuttalliosis due to *Nuttallia equi* Laveran (1901), França (1909). Dschunkowsky and Luhs (1913) moreover have since reported that horses and mules in Transcaucasia may become infected either with *Nuttallia equi* or *Piroplasma caballi*.

The cases recorded below were all in-patients at the Madras Veterinary Infirmary. There were several cases of which no mention is made in this paper, but in which the disease was verified by microscopical examination, the infection being due to either *P. caballi* or *N. equi.*

Case 1 (Nuttalliosis). Since the two species of parasites above referred to were differentiated and classified, probably the first observation in India, here recorded, was made on a Gelding, the property of Mr G. F. Paddison, I.C.S. The horse was admitted into the Madras Veterinary College Hospital as an in-patient for treatment for biliary fever on 19th September 1912. It appears the horse had been taken to various parts of the Presidency, so that it was difficult to determine the precise place at which the animal became infected. It was ascertained that the animal was frequently inclined to be sluggish in its movements for short periods of time but this condition evidently had no bearing on the disease.

The symptoms observed at the Infirmary were briefly as follows:— the conjunctival mucous membranes, at first of a pale yellow colour, became subsequently hyperaemic with serosanguinolent infiltration. At a later stage well marked petechiae were noticed on the membrana nictitans. The appetite was irregular, the animal now and again eating a little corn and grass. Dulness was noticed from the beginning; the animal presented appearances of weakness and had an unsteady gait almost terminating in paraplegia before death. Haemoglobinuria was observed on the last seven days. Fever was present, a maximum of 107° F. was recorded on 30th September in the evening; it fluctuated slightly to the time of death, at 6 p.m. on 7th October 1912.

The parasites in the peripherial blood were first noticed on the morning of 30th September. They were found in large numbers and included the spherical, piriform and rosette varieties. All these forms could be distinguished fairly easily from the start and they increased in number as death approached.

The *Autopsy* revealed several morbid changes: well marked general bile staining of the subcutaneous tissue; liver considerably enlarged, jaundiced, weight 20 lbs.; spleen enlarged, weight 9 lbs.; kidneys much enlarged, congested and icteric, each kidney 2 lbs.; heart enlarged, contained hardly any blood.

Subinoculation Experiment. Previous to death two horses were inoculated (2. x. 1912) from the blood of horse 1, 18 c.c. into an aged C. B. Gelding, and 20 c.c. into an aged Waler Mare, both intravenously. The result was negative.

Case 2 (Nuttalliosis). The next case, belonging to Mr Rice, I.C.S., was a horse sent to the Madras Veterinary College Hospital from Anantapur, a station 350 miles from Madras. It had done a great deal of touring. The owner wrote to say that the horse was quite well till 17th November when it suddenly refused food. While encamped at a different place on the 18th, the animal was infested with *ticks*, and it is suspected that these also attacked the animal in camp during November 14–16th. The animal was marched to Anantapur, where a Veterinary Assistant took its temperature which read 104° F. The temperature however gradually went down under treatment, became normal and remained so for a long time, but it rose again to 101° F. on 1st December, and it was sent to Madras, on 2nd December 1912.

Blood examination revealed an infection of *Nuttallia equi*. The animal was discharged on 27th January 1913. Several specimens of *Hyalomma aegyptium* were picked from this animal.

Case 3 (Nuttalliosis). The next case occurred in Brown Waler Gelding (Cob), 6 years old, belonging to the Principal of the Madras Veterinary College and was admitted as an in-patient for treatment on 25th February 1913. The animal was dull, off its feed; temperature 104° F.; mucous membranes, light yellow in colour, and bearing a few red spots. For the following four days, the temperature varied between 103° and 105·4° F., after which it became normal. The urine was slightly coloured for a day or two after admittance, but subsequently cleared up. The parasites were of the rosette, piriform and round forms, and, except on one day, it took some searching to find them. The animal was discharged on 10th March 1913.

Cases 4–7 (Nuttalliosis). Four Waler horses belonging to the Madras General Post Office were under treatment at the hospital at various times during the months of April, May and June 1913. These cases were microscopically confirmed as Nuttalliosis. The animals were duly discharged after treatment but were never good at their work subsequently.

Case 8 (Piroplasmosis). B. W. Gelding, belonging to Miss Miller of Royapuram, Madras, admitted on 16th November 1912, for biliary fever. The animal was ailing about four days previous to admission to the Veterinary Hospital. The animal was in fairly good condition; temperature 104° F., with yellowness of the visible mucous membranes; it was slightly off its feed.

Microscopical examination revealed *P. caballi* in the blood on six days after admission; they were sparse and of the ring or piriform type. On an average there were about six infected corpuscles in the five

films that were examined on each of the six days. This animal recovered after medicinal treatment, which consisted principally of quinine and salol and was discharged from the Hospital on 9th December 1912.

It was ascertained that this animal had never been out of Madras during about four years since it had been in the possession of the present owner. A month or so before it became ill it was sent for a few days to a local stable and, on its return to its home, ticks were removed from various parts of the body, which were immediately destroyed. I saw ticks on several animals standing in this stable which I visited subsequently. These ticks belonged to the species *Hyalomma aegyptium*. Frequently on animals admitted into the Veterinary Hospital for treatment from this stable, I discovered ticks belonging to the same species. Several of them were dissected but with negative results.

Subinoculation experiment: an aged W. Gelding was inoculated intravenously with blood (5 c.c.) from Case 8 with positive result. The parasites (*P. caballi*), after an incubation of 12 days, were easily distinguished in the peripheral blood, ring forms and bigeminum forms were mainly encountered. No medicinal treatment was given and the animal died early on the morning of 7th December 1912. There were the usual symptoms of piroplasmosis: depression, rise of temperature to 106° F., yellowness of the visible mucous membrane and petechiae on the membrana nictitans, unsteady gait with complete paraplegia the evening before death. It should be stated however that this animal showed slight loss of co-ordination in the hind quarters before inoculation.

Autopsy: characteristic; general bile staining of the subcutaneous tissue; liver enlarged, weight 17½ lbs.; spleen greatly enlarged, somewhat pulpy, weight 13 lbs.; heart enlarged, brownish in colour, weight 3 lbs.

Second subinoculation experiment: the aged Waler Mare already referred to in which the inoculation of *Nuttallia equi* strain from Case 1 had failed, was inoculated with 5 c.c. blood from Case 8 on 5th December 1912, but showed no reaction.

Case 9 (*Piroplasmosis*). This case was discovered at the Madras Veterinary College Hospital in a Ch. W. Gelding belonging to Mr C. Morrison of Madras. This animal was admitted on 22nd December 1912, for a capped elbow. On 9th January 1913 it had an attack of colic from which it recovered. On 14th January the temperature rose to 105° F., and blood examination revealed an infection of

P. caballi. The animal was discharged cured after medicinal treatment on February 1913.

Subinoculation experiment: before this animal left the Hospital an aged C. B. pony, was inoculated with 10 c.c. of blood of Case 9, but with negative results.

It may be mentioned here that all the animals used for subinoculation experiments were past work and were sent to the Hospital to be destroyed. It was only possible to carry on experiments upon such animals which had evidently been a long time in the country and probably owed their seeming insusceptibility to the fact that they were already infected.

Nuttalliosis seems to be a more severe form of infection than Piroplasmosis, and relapses are likely to occur in the former disease. The latter takes a mild course and is more amenable to treatment. One characteristic feature noticed in fatal Nuttalliosis is that the bright red spots seen on the conjunctival mucous membrane alter to blotches, and after a few days may coalesce to form large patches. On the other hand in some cases of Piroplasmosis a few bright red petechial spots occur on the conjunctiva, but these red spots have no tendency to increase in size and change in colour as they do in cases of Nuttalliosis.

Theiler (1906) working with equine piroplasmosis in South Africa states that in cases of recovery from the first attack of biliary fever (Nuttalliosis?) a second attack is usually observed during which the parasite is less frequently found than in the first attack, and also that the parasites change in shape, usually assuming the leaf form. Mr Rice's horse (Case 2, Nuttalliosis) above referred to, after being discharged on 27th January 1913, was readmitted on 12th March 1913, with what was clinically the same disease, but which turned out to be a mild attack, readily passing off in the course of seven or eight days. The four horses (Cases 4–7 Nuttalliosis) belonging to the General Post Office were other cases which come under the same category. These animals had repeated attacks of fever. The organisms were demonstrable in the blood, but were scanty and somewhat modified in form. One of the animals was under treatment for quite a long time at the beginning of the year 1913.

In the article on equine biliary fever in India by Jolliffe (1907) the writer does not seem to agree with the statement of Pallin (1905) that the death rate from this cause is low and that the disease has a tendency towards recovery. Jolliffe's experience indicates that in a single outbreak he had a mortality of over 35%. It is probable that both these

writers are correct and that the discrepancies in their results were due to their dealing with different parasites, viz. *P. caballi* and *N. equi*.

Concerning the mode of propagation of this disease, ticks came to be regarded with a great deal of suspicion, and personally I have noticed all the cases that have come under my observation harbouring these acarids at one time or another on their bodies. This suspicion is accentuated by the fact that in an outbreak of equine biliary fever in Southern Russia (1908), ticks recognised as *Hyalomma aegyptium* were found on the horses, although it has not yet been proved that this tick is the transmitting agent. Joliffe (1907) has observed in outbreaks of biliary fever amongst cavalry horses in India, the absence of ticks and suggests that some winged insect may possibly be concerned in the dissemination of the parasites. Lingard (1904) assumed that the mosquitoes were the carriers in this country.

Marzinowsky and Bielitzer (1908), working on equine piroplasmosis *P. caballi* in Russia, found the tick *Dermacentor reticulatus* upon infected horses. They state that locally bred horses scarcely suffer from this disease and appear immune; on the other hand imported horses are very susceptible and often die. Theiler (1905) demonstrated equine biliary fever to be a tick-borne disease in South Africa, the carrier being *Rhipicephalus evertsi*. As neither *Dermacentor reticulatus* nor *Rhipicephalus evertsi* has yet been found in India to my knowledge, the intermediate host still remains to be discovered in this country. This discovery may prove of practical importance in the treatment of the disease in horses. For the present quinine and salol appears to be the best treatment available[1], as evidenced from the satisfactory results obtained by means of these drugs at the Madras Veterinary College Hospital.

Bielitzer's (1910) recommendation that all young equine stock should be exposed to tick infection, thereby acquiring a natural immunity, may be mentioned. Another method of immunisation is that adopted by Theiler (1906). He took the serum of immune, *i.e.* recovered donkey foals, and inoculated it subcutaneously into clean animals. He found there were fewer deaths when blood was taken in this way than when taken from a horse foal. These methods have been recommended for many years in bovine piroplasmosis.

[1] There was a severe outbreak of biliary fever in horses at Nagpur in October—December 1913, in which the two species of parasites, *N. equi* and *P. caballi*, were recorded. Two valuable English race horses, both infected with *Nuttallia equi*, the property of Mr Fagan (Personal Assistant to the Inspector General of Police, Central Provinces), were successfully treated with *trypanblue* alone.

Both *N. equi* and *P. caballi* occur in equine biliary fever in India, giving rise to two distinct diseases in the Madras Presidency. The infection is more prevalent among Australian horses. Both forms are amenable to treatment, if diagnosed at an early stage, but Nuttalliosis may persist and is liable to recurrence when the infected animal is subjected to unfavourable conditions.

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"TICK PARALYSIS" IN MAN AND ANIMALS.

FURTHER PUBLISHED RECORDS, WITH COMMENTS.

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Since the appearance of the papers by Hadwen and myself dealing with the subject of "Tick Paralysis[1]," I have had my attention drawn to two interesting papers. The reference to the first paper, by Borthwick (1905), was found whilst compiling Part II of the *Bibliography of the Ixodoidea*, and secondly, I am indebted to the author, Dr I. U. Temple, of Pendleton, Oregon, for sending me a copy of his valuable contribution. The latter appeared in a journal which will scarcely be accessible to European readers, and I therefore have no hesitation in reprinting the greater part of it below. The following account is condensed from the paper by Borthwick (VII. 1905, pp. 33–35).

"Tick Paralysis" in Sheep in Cape Colony.

Borthwick states that "Tick Paralysis" occurs over a large area of Cape Colony, and that the affection has for many years been recognised by sheep farmers there who unanimously attribute it to the attacks of *Ixodes pilosus*. The disease had prevailed to an unusual degree during the preceding winter on certain specified farms on the Fish River Rand and in Somerset East District. The affection occurs most commonly in May–June on sheep runs in long and bushy veld.

Symptoms. At first the affected sheep, whilst grazing, are observed to lag behind the rest of the flock; they lie down, but rise and walk

[1] See Hadwen (x. 1913). *Parasitology*, VI, 283—297; Hadwen and Nuttall. *Ibid.*, 299—301, where references to earlier authors will be found on p. 297.

after the flock when approached. After a short time the animals no longer rise on being approached; if they are lifted up they walk unsteadily and soon lie down again. The sheep may be completely paralyzed within six hours after the onset of symptoms, and they appear as if asleep for 24–48 hours; recovery follows rapidly in most cases. Very few animals die if they are left undisturbed in a shed, but deaths are fairly common in the open, for the prostrate sheep fall victims to marauding jackals. The affected sheep show no febrile reaction.

Autopsy. No noticeable lesions were observed.

Inoculation experiments gave negative results. Veterinary Surgeon Spreull failed twice to transmit the affection from paralysis sheep to healthy sheep by blood inoculation.

Ticks and their bearing on the affection. The ticks, *Ixodes pilosus*, were found both on healthy and affected animals. "The number of ticks does not seem to influence the severity of the case, two ticks inducing as bad a case as fifteen; but if the ticks are removed immediately the sheep is observed to be sick, the recovery is more rapid, within 12 hours, in many cases, recovery is complete."

I. pilosus attaches itself to the host mainly inside the thighs, behind the elbows and between the lower jaws.

Effect of Dipping. The use of Cooper's Dip was found to be effective. Of 88 sheep grazed in a kloof where the disease prevailed, 68 were dipped and 20 were not dipped. During three weeks no cases of tick paralysis occurred among the 68 dipped animals, whilst 6 cases (3 fatal) occurred among the 20 undipped sheep. Moreover, at Hartmanskraal, in the month of June, where 60–70 cases occurred, the trouble ceased on two occasions when dipping was resorted to.

Tick Paralysis in Man in Oregon.

The following extract is from the interesting paper by Temple (IX. 1912, pp. 508–514) with but slight alterations in the numbering of the cases consecutively, typographical corrections and a word or two added in [ ] brackets. Dr Temple writes:—

"The following cases have come under my observation while practising in Wallowa and Umatilla counties, Oregon:

Case 1. Little girl, Thompson, of Wallowa, Oregon. Age five, retired at bed-time in her usual normal health; all her life she had been in perfect health. Upon rising the following morning she was

unable to stand on her feet; in attempting to stand she would fall in a heap on the floor. She had perfect control of her upper limbs, temperature normal, absence of pain. The child would have been considered well had it not been for the impediment in standing. I thought, however, the symptoms indicated a serious lesion. She was given calomel and strychnine.

The next morning I called, to find her completely recovered, much to my surprise. The mother told me she had removed a *tick* from the back of the girl's head. I attached no importance to this, thinking it only a coincidence, and concluded the medicine had done wonders. I had not at this time heard of Tick Bite producing paralysis.

Case 2. Little girl, Aiken, age six, of Paradise, Oregon, was brought to my office, a distance of forty miles, lying upon a cot in a spring waggon. Her symptoms were as follows: upon retiring the previous day she was in her usual normal health. The following morning upon arising she was unable to stand upon her feet, according to the parent's story. Upon arriving at my office I found paralysis extending to the knees; slight temperature, no pain, sensory nerves normal, motor nerves completely paralyzed, reflexes absent. She was placed in bed, given purgatives, Potassium Iodide, etc.

The following day the paralysis had extended to the upper limbs, and before night of the third day to the nerves of the throat (Hypoglossal). Thorax and larynx were involved. Her breathing was laboured, she was unable to swallow liquids, phonation was impossible, and she could make only low, guttural sounds.

At the evening call I directed the nurse to apply tincture of Iodine to the spinal column the entire length, and in doing so she found two *ticks* fully distended with blood over the junction of the spinal column with the occipital bones in the hollow depression. I was called by telephone and examination confirmed the report. I removed them by the application of undiluted creoline. I fully expected the child to die. To my surprise, the following morning she was very much improved. By evening she was able to speak, the paralysis gradually receded, remaining longest in the feet. At the end of one week she was able to return to her home.

Case 3. Little girl, age 10 (name Chauvette), at Enterprise, Oregon. I was consulted over the telephone, and the symptoms described being similar to those in previous cases, I directed the mother to search the child for ticks. She did, but failed to find them. When I was called, I had the child stripped, and noted the following symptoms: the patient

was nervous, temperature low, pulse rapid, knee reflexes absent, and the child was unable to stand upon her feet. I told the mother the child had symptoms of Tick Paralysis, and we would again attempt to locate the tick.

A careful search located a single *tick* at the vertex, slightly to one side of the part of her hair. The tick was removed under antiseptic precautions. The child had completely recovered the day following its removal. No other treatment was used.

Case 4. Little girl, aged 4, name Welch, of Ukiah, Umatilla county. At this time I was practising at Pendleton, Oregon. I was called by telephone from a distance of forty miles. The little girl's symptoms were described to me as follows by the father. The child had seemed well the day previous, but at this time she was unable to stand upon her feet; she complained of no pain, seemed well so far as the father could see, except that she had lost control of the lower limbs. I directed the father to return to his home, search the child carefully for ticks, especially upon the head, with instructions to notify me of the results of the search. He returned within two hours and informed me that the *ticks* had been located, two in number, on the back of the child's head. He had removed them according to my instructions with kerosene. The father reported the following day that the child was very much better and a prompt recovery ensued, thus avoiding a forty dollar doctor bill by telephone consultation. No other treatment was used.

Of the four cases herewith reported, three were from Wallowa county and one from Umatilla county. No two from any immediate locality and being separated probably 200 miles, but all in the range of the Blue mountains of Eastern Oregon.

It is also noteworthy that the ticks are usually located in pairs; this being for the purpose of fertilizing the ovum and that the principal location is in the hair of the head.

Case 5. The following report comes from Dr G. W. Gregg, Ashland, Oregon. He says: 'In reference to ticks I have seen but one case of paralysis from tick bite. Have seen at least two others that presented rather severe local infection, but no paralysis. The case in point occurred about twelve years ago, while I was practising at Wallowa, Oregon. I was called about six miles north of Wallowa to see a little girl, age seven, whom the parents had informed me had about three days previously complained of tingling and slight numbness in the fingers of both hands, which soon extended to the toes, then gradually

extending up the arms and legs and was gradually accompanied by loss of motion.

The morning I was called the tongue and throat were also affected, the child was unable to talk distinctly and could not swallow. Was rational all the time. On examination, I found paralysis of motion of the arms and legs, sensation present in the limbs, pupils dilated, temperature sub-normal, expression dull and patient inclined to somnolence. I found a big fat *tick* about the size of a large bean attached about two inches posterior to the right ear in the hair. I removed the tick antiseptically and left a few doses of strychnine. In two hours there was improvement in her condition which continued, when in six or seven days the child was completely recovered. (Signed) G. W. Gregg.'

In this case you will observe the initial symptoms were described by her parents as beginning in the hands, then in the feet. I feel confident this is not correct, as reports from the laity are not always dependable. It is my opinion the symptoms began in the feet.

The following interesting reports come from Dr E. C. Kirby, of Elgin, Oregon:

'*Case* 6. Baby K., age 2, July, 1913. *Tick* bite on right shoulder, not found or removed until the patient was nearly dead. Temperature 104°, complete paralysis, protruding tongue, spotted, mottled and reddish condition of entire body, duration three days; death followed.

Case 7. Willie C., age 4, residing near the summit of Blue Mountains, in wood camp; temperature 104°, marked paralysis of tongue and jaw, tongue thick and protruding. Paralysis as a whole complete, reflexes absent.

Patient had been sick about four days before I saw him. Skin mottled, purplish, patient removed to the hospital, became unconscious and died. Location of *tick* on right temple bone.

Case 8. Willie Burger, age 7; first complained for several days of being ill, the walking wobbly, eyes became fixed. When I was called I found *tick* attached to nape of neck; removed by excision; pulse very rapid; patient did not seem alarming; patient grew worse until he could not stand; falling backward when he fell. Put him to bed for three weeks; recovery after six weeks.

Case 9. James M., age 5, taken sick with chill and high fever. I was called in on same day. *Tick* located on right ear. Temperature 104°, no paralysis; tick removed and patient made rapid recovery.

I have had many tick bites among adults that cause ugly indolent ulcers; have also had abscesses from the bites. (Signed) E. C. Kirby.'

I mention this last case to indicate to you the different effects produced by the bite of the tick. I presume there is not a physician who has practised in Eastern Oregon a great length of time who has not had severe ulcers from the infection of the tick bite.

It is my opinion the different effect lies in the fact that there are such a variety of ticks inhabiting the Eastern Oregon region.

The following interesting report comes from Dr E. T. Anderson, now of Weiser, Idaho. I preface this report by saying that Dr Anderson was my successor in practice at Enterprise, Oregon, and of necessity covering the same field from which my first three cases are reported:

'*Case* 10. E. A., Sept. 3rd, 1908; male, age 8; was in normal health the evening before, in the morning was unable to stand upon his feet, progressive motor paralysis, ascending in character, sphincters involved, no pain; temperature 102 $\frac{3}{5}$°, pulse 110, respiration 32, reflexes absent in lower extremity. Examination revealed two *ticks*, one large tick distended with blood overlying one small one [doubtless ♀ and ♂] attached to the nape of the neck, within the hair.

Removal of tick was followed by rapid recovery. No other treatment instituted. Recovery complete in three days. (This was the doctor's own little son. Author.)

Case 11. Bessie B., June 7, 1909, age 13. Reported by telephone. The symptoms of acute ascending paralysis was described. I instructed them to look for *ticks*, the same being found over posterior scalp and removed. The child promptly recovered from the paralysis, but from the seat of attachment a large burrowing abscess formed, very indolent in character. I treated this with irrigation (antiseptic) and painted with silver nitrate solution 10 per cent, with complete recovery. (Doubtless in this case the tick's head [capitulum] was not removed and a severe local infection followed. Author.)

Case 12. Bessie J., May 4, 1910; age 2 years, 3 months. Patient moribund when first seen, dying within the hour thereafter; the evening before apparently in good health. When first seen presented picture of complete motor paralysis, with relaxation of the sphincters, pulse 60, temperature 97°, respiration 46, tongue protruding, phonation absent and unable to swallow, reflexes absent. Death. One single *tick* attached at base of occiput.

Case 13. Willie R., June 15, 1909; male, age 11. Complained of weakness for two days, morning of third day could not stand upon feet; by noon of the same day paralysis had extended to hips and involved the sphincters. By evening hands and arms involved. First seen at 11 p. m., when muscles of throat were also paralyzed, tongue protruding, druling excessive, temperature 99°, pulse 84, respiration 47, pulse soft and weak. Examination revealed one large *tick* distended with blood attached near the crown of the head. Two hours after removal the symptoms of the throat were improved, respiration 32. By morning temperature 98·6°, pulse 74, respiration 20, paralysis in feet and lower limbs, phonation possible with a complete recovery in four days. (Signed) E. T. Anderson, Weiser, Idaho.'

It is apparent that death in case No. 12 was due to the age of the child, with little resistance. Case No. 13 shows all the typical symptoms of the ascending character of the paralysis and describes most clearly the process of invasion and recovery.

I have had considerable correspondence with staff members in the Department of Entomology, Washington, D. C., and from such correspondence and the bulletins sent to me I note there is a great variety of ticks infesting our western slope, and also from this particular region. I have sent three ticks from Eastern Oregon...

The progress of paralysis is apparently quite rapid, being from 3 to 5 days from time of beginning to a complete paralysis and often death. Suffice to say the treatment is the removal of the tick by application of coal oil (petroleum), or creoline, which loosens the hold. The prognosis is favourable if paralysis has not advanced to the stage of affecting the organs of respiration or the heart.

In conclusion, I acknowledge that the reports herewith are not classical in their character, and much of the report is taken from memory, that there is apparently considerable difference in the description and symptoms as given by Drs Gregg, Kirby and myself, but in the main features we are of one opinion, namely:

That there are certain species [of tick] capable of producing complete paralysis, of an ascending character, and also death.

That the motor nervous system is affected and that the sensory nerves are not disturbed.

That it does not produce any changes in the brain or cord as recovery is rapid and complete when the tick or ticks are removed in the early stage of the paralysis and a local irritation seems to be the predominating feature.

That the cases herewith reported are sufficient to warrant investigation by the Department of Entomology, and are worthy of mention in North-west medical literature."

Remarks upon the foregoing records.

(1) *Tick paralysis in Sheep.*

The paper by Mally (1904), cited by Hadwen, and that of Borthwick (1905), here abstracted, attribute a disease called "Tick paralysis" in sheep in Cape Colony to the animals being attacked by *Ixodes pilosus* Koch. Both authors state that farmers in Cape Colony have no doubt whatever as to the bearing of the tick upon the affection; the use of Cooper's dip has served to check the disease in paralysis flocks and to prevent the affection in animals exposed in localities where it prevails. The disease has a seasonal incidence, it is acute in its onset, recovery occurs rapidly in most cases. Fever is absent, and the disease is not communicable by blood inoculation. Recovery is hastened by the removal of the ticks. In animals that have died from the disease no noticeable pathological lesions are observable.

In the absence of further data, these observations which I have thus briefly epitomised, could not be regarded as more than merely suggestive. The important paper by Hadwen (*Parasitology*, VI, 283) has, however, thrown much light upon the subject. He not only observed a practically identical disease in sheep in British Columbia, but he reproduced it experimentally by means of ticks (*Dermacentor venustus* Banks).

(2) *Tick Paralysis in Man.*

That a similar affection appears to occur in other animals in British Columbia has been indicated already by Hadwen (*Parasitology*, VI, 285), and Hadwen and Nuttall (*Ibid.*, p. 298) reproduced the disease in a dog in Cambridge, in July, 1913, the ticks (*D. venustus*) having been collected, 5, VI. 1913, from the human subject in the mountains near Nelson, British Columbia. Hadwen (p. 295) quotes Todd (1912) with regard to cases of tick paralysis occurring in five children and one adult in British Columbia, it being assumed that *D. venustus* was the "tick" concerned; he also cites Eaton (1913) with regard to a case in a child in Australia (tick undetermined); the latter author, moreover, quotes cases which have been reported from that country by Cleland.

Since we have been able to reproduce tick paralysis experimentally there can no longer be any doubt as to the existence of this disease.

The symptoms described in the human subject agree with those observed in experimental cases in the sheep and dog, and the symptoms noted in cases occurring in sheep in the field. The 13 cases recorded by Temple in Oregon are of great interest, and in their symptomatology appear in most of the cases to agree fully with the cases briefly reported by Todd from British Columbia.

It is to be regretted that we have not more precise data as to the species of ticks concerned in the causation of the human cases. Todd and Temple merely refer to "ticks." It is true that Temple sent "three ticks from Eastern Oregon" to F. C. Bishopp and W. D. Hunter for determination, and that they proved to be *Dermacentor albipictus, D. venustus* and *Ornithodorus megnini*[1] respectively. The last-mentioned species of tick has probably nothing to do with the case, and it is not stated where and how the other two ticks were collected. It is suggestive, however, in view of the experimental results, previously cited, that *D. venustus* is found in Oregon where Temple's cases occurred.

Having formerly lived for a number of years in the Western United States and collected many ticks from my person on expeditions into the mountains, I may say that it was my custom to take a fine comb with me and to remove the ticks which so frequently attached themselves in the hair at the back of the head and neck. I still have some mounted specimens of *Dermacentor* collected in this manner as long ago as 1879 in California. It was a frequent occurrence after a day's shooting in the brush to remove several ticks with the comb. Too much importance might, therefore, be attached to the finding of a tick or two upon the head in children suffering from supposed tick paralysis, for it is reasonably certain that ticks would be frequently found on normal persons if they were looked for with equal diligence. The cases reported by Todd and by Temple, in which ticks were found attached to the head, might well be explained as due to mere coincidence.

That we are dealing with a definite affection, however, has been rendered certain by the experimental results obtained by Hadwen on sheep and by Hadwen and Nuttall on the dog.

No particular species of tick appears to be concerned in the causation of tick paralysis, since *Dermacentor venustus* in British Columbia (? and Oregon) and *Ixodes pilosus* in South Africa both

[1] This tick, the well-known "spinose ear tick" of American authors, has not been found before so far to the north as Oregon; it was found in a man's ear, its usual site of attachment to a host.

appear capable of inducing the same affection. It is clear that further investigation is required to elucidate the nature of the disease and to determine what species of ticks are capable of producing it. For the present we only possess clear experimental evidence implicating *Dermacentor venustus*.

REFERENCES.

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